PCT

INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 31/35, 31/05, 31/375

A1

(11) International Publication Number: WO 99/18953

(43) International Publication Date: 22 April 1999 (22.04.99)

(21) International Application Number: PCT/US98/21887

(22) International Filing Date: 16 October 1998 (16.10.98)

(30) Priority Data:
08/951,912 16 October 1997 (16.10.97) US

(71) Applicant: CHILDREN'S HOSPITAL OAKLAND RESEARCH INSTITUTE [US/US]; 747 Fifty Second Street, Oakland, CA 94609–1809 (US).

(72) Inventors: FISCHER, Horst, Bernhard; 839 Carmel Avenue, Albany, CA 94706 (US). ILLEK, Beate; 839 Carmel Avenue, Albany, CA 94706 (US).

(74) Agents: MAKI, David, J. et al.; Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104–7092 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: FLAVONOIDS FOR CYSTIC FIBROSIS THERAPY

(57) Abstract

Compositions and methods for therapy of cystic fibrosis and other conditions are provided. The compositions comprise one or more compounds such as flavones and/or isoflavones capable of stimulating chloride transport in epithelial tissues. Therapeutic methods involve the administration (e.g., orally or via inhalation) of such compositions to a patient afflicted with cystic fibrosis and/or another condition responsive to stimulation of chloride transport.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

FLAVONOIDS FOR CYSTIC FIBROSIS THERAPY

TECHNICAL FIELD

The present invention relates generally to the treatment of cystic fibrosis. The invention is more particularly related to compositions comprising one or more compounds such as flavones and/or isoflavones, which may be used to activate chloride transport (*i.e.*, absorption and/or secretion) in epithelial tissues of the airways, the intestine, the pancreas and other exocrine glands, and for cystic fibrosis therapy.

BACKGROUND OF THE INVENTION

Cystic fibrosis is a lethal genetic disease afflicting approximately 30,000 individuals in the United States. Approximately 1 in 2500 Caucasians is born with the disease, making it the most common lethal, recessively inherited disease in that population.

Cystic fibrosis affects the secretory epithelia of a variety of tissues, altering the transport of water, salt and other solutes into and out of the blood stream. In particular, the ability of epithelial cells in the airways, pancreas and other tissues to transport chloride ions, and accompanying sodium and water, is severely reduced in cystic fibrosis patients, resulting in respiratory, pancreatic and intestinal ailments. The principle clinical manifestation of cystic fibrosis is the resulting respiratory disease, characterized by airway obstruction due to the presence of a thick mucus that is difficult to clear from airway surfaces. This thickened airway liquid contributes to recurrent bacterial infections and progressively impaired respiration, eventually resulting in death.

In cystic fibrosis, defective chloride transport is generally due to a mutation in a chloride channel known as the cystic fibrosis transmembrane conductance regulator (CFTR; *see* Riordan et al., *Science 245*:1066-73, 1989). CFTR is a linear chloride channel found in the plasma membrane of certain epithelial cells, where it regulates the flow of chloride ions in response to phosphorylation by a cyclic AMP-

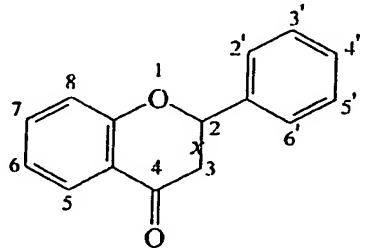
dependent kinase. Many mutations of CFTR have been reported, the most common of which is a deletion of phenylalanine at position 508 (ΔF508-CFTR), which is present in approximately 70% of patients with cystic fibrosis. A glycine to aspartate substitution at position 551 (G551D-CFTR) occurs in approximately 1% of cystic fibrosis patients.

Current treatments for cystic fibrosis generally focus on controlling infection through antibiotic therapy and promoting mucus clearance by use of postural drainage and chest percussion. However, even with such treatments, frequent hospitalization is often required as the disease progresses. New therapies designed to increase chloride ion conductance in airway epithelial cells have been proposed, but their long term beneficial effects have not been established and such therapies are not presently available to patients.

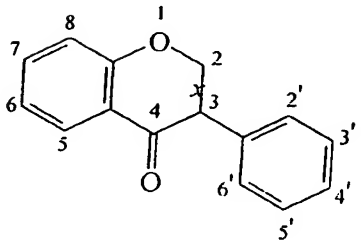
Accordingly, improvements are needed in the treatment of cystic fibrosis. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for enhancing chloride transport in epithelial cells and for the therapy of cystic fibrosis. Within one aspect, the present invention provides methods for enhancing chloride transport in epithelial cells, comprising contacting epithelial cells with a compound selected from the group consisting of flavones and isoflavones, wherein the compound is capable of stimulating chloride transport and wherein the compound is not genistein. Within certain embodiments, the compound is (a) a polyphenolic compound having the general formula:



or



wherein carbon atoms at positions 2, 3, 5, 6, 7, 8, 2', 3', 4', 5' and 6' are bonded to a moiety independently selected from the group consisting of hydrogen atoms, hydroxyl groups and methoxyl groups, and wherein X is a single bond or a double bond; or (b) a stereoisomer or glycoside derivative of any of the foregoing polyphenolic compounds. Such compounds include, within certain embodiments, quercetin, apigenin, kaempferol, biochanin A, flavanone, flavone, dihydroxyflavone, trimethoxy-apigenin, apigenin 7-O-neohesperidoside, fisetin, rutin, daidzein and prunetin. For enhancing chloride transport in airway epithelial cells of a mammal, compounds may be administered orally or by inhalation. Other epithelial cells that may be employed include intestinal, pancreas, gallbladder, sweat duct, salivary gland and mammary epithelial cells. Within certain embodiments, the compound is combined with a substance that increases expression of a CFTR; and/or a chemical chaperone that increases trafficking of a CFTR to the plasma membrane.

Within other aspects, methods for enhancing chloride transport in epithelial cells may comprise contacting epithelial cells with a compound selected from the group consisting of reservatrol, ascorbic acid, ascorbate salts and dehydroascorbic acid. Such compounds may further be used in combination with a flavone or isoflavone as provided above.

Within other aspects, the present invention provides methods for treating cystic fibrosis in a patient, comprising administering to a patient a compound as described above, wherein the compound is capable of stimulating chloride transport. Within certain embodiments, the compound is genistein, quercetin, apigenin, kaempferol, biochanin A, flavanone, flavone, dihydroxyflavone, trimethoxy-apigenin, apigenin 7-O-neohesperidoside, fisetin, rutin, daidzein or prunetin. Within other embodiments, the compound is reservatrol, ascorbic acid, ascorbate salts and

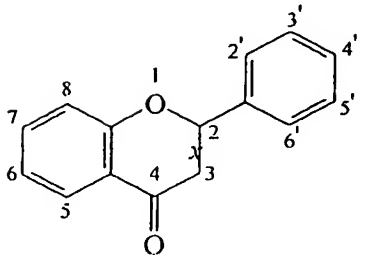
dehydroascorbic acid. Such compounds may be administered alone or in combination. Compounds may be administered orally or by inhalation. Within certain embodiments, the compound is combined with a substance that increases expression of a CFTR; and/or a chemical chaperone that increases trafficking of a CFTR to the plasma membrane.

Within further related aspects, the present invention provides methods for increasing chloride ion conductance in airway epithelial cells of a patient afflicted with cystic fibrosis, wherein the patient's CFTR protein has a deletion at position 508, the method comprising administering to a mammal one or more compounds as described above, wherein the compound is capable of stimulating chloride secretion in the airway epithelial cells.

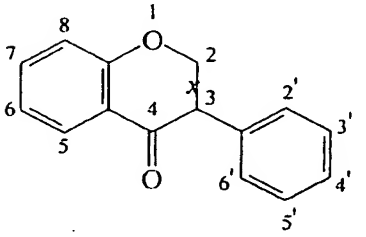
Within still further related aspects, the present invention provides methods for increasing chloride ion conductance in airway epithelial cells of a patient afflicted with cystic fibrosis, wherein the patient's CFTR protein has a mutation at position 551, the method comprising administering to a mammal one or more compounds as described above, wherein the compound is capable of stimulating chloride secretion in the airway epithelial cells.

Within further aspects, pharmaceutical compositions for treatment of cystic fibrosis are provided, comprising (a) one or more flavones or isoflavones capable of stimulating chloride transport and (b) one or more of: (i) a compound that increases expression of a CFTR in an epithelial cell; and/or (ii) a chemical chaperone that increases trafficking of a CFTR to a plasma membrane in an epithelial cell; and; and in combination with a pharmaceutically acceptable carrier. Within certain embodiments, the flavone or isoflavone may be genistein, quercetin, apigenin, kaempferol, biochanin A, flavanone, flavone, dihydroxyflavone, trimethoxy-apigenin, apigenin 7-O-neohesperidoside, fisetin, rutin, daidzein and/or prunetin, in combination with a pharmaceutically acceptable carrier.

Within still further aspects, a pharmaceutical composition for treatment of cystic fibrosis may comprise: (a) a polyphenolic compound having the general formula:



or



wherein carbon atoms at positions 2, 3, 5, 6, 7, 8, 2', 3', 4', 5' and 6' are bonded to a moiety independently selected from the group consisting of hydrogen atoms, hydroxyl groups and methoxyl groups, and wherein X is a single bond or a double bond; or a stereoisomer or glycoside derivative of any of the foregoing polyphenolic compounds; (b) a compound selected from the group consisting of reservatrol, ascorbic acid, ascorbate salts and dehydroascorbic acid; and (c) a physiologically acceptable carrier.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a recording of transepithelial short-circuit current (Y axis) as a function of time (X axis), showing the effect of apigenin on the current across a Calu-3 cell monolayer. Measurements were performed in an Ussing chamber, where the basolateral membrane was permeabilized with α-toxin and a chloride gradient was applied across the apical membrane as a driving force. Tissue was first stimulated with cAMP (100 μM). Apigenin (50 μM) was subsequently added as indicated. The horizontal bar represents 100 seconds, and the vertical bar represents 12 $\mu A/cm^2$.

Figure 2 is a recording showing the effect of quercetin on transepithelial short-circuit current across a Calu-3 cell monolayer in an Ussing chamber, where the basolateral membrane was permeabilized with α-toxin and a chloride gradient was applied across the apical membrane as a driving force. Tissue was first stimulated with cAMP (100 μM). Quercetin (30 μM) was subsequently added as indicated. Bars are 140 seconds (horizontal) and 12 $\mu A/cm^2$ (vertical).

Figure 3 is a recording illustrating the dose-dependent stimulation of transepithelial chloride secretion by quercetin (in the amounts indicated) across a primary bovine tracheal epithelium. Amiloride (50 μM) was added to block sodium transport as indicated. The CFTR channel blocker diphenylcarboxylate (DPC, 5 mM) was added as shown.

Figure 4 is a recording showing the effect of biochanin A on transepithelial short-circuit current across a Calu-3 cell monolayer in an Ussing chamber, where the basolateral membrane was permeabilized with α-toxin and a chloride gradient was applied across the apical membrane as a driving force. The tissue was first stimulated with forskolin (Fsk, 10 μM). Subsequent addition of biochanin A (Bio, 100 and 300 μM) was subsequently added as indicated.

Figure 5 is a cell-attached single channel patch clamp recording from a 3T3 cell expressing ΔF508-CFTR. The cell was treated with 10 μM forskolin as shown. Genistein (50 μM) and apigenin (50 μM). were added where indicated by boxes. The holding potential was 75 mV. and channel openings were upward.

Figure 6 is a whole cell patch clamp recording on an airway epithelial cell homozygous for ΔF508-CFTR. Before the measurement, the cell was incubated for 2 days in 5 mM 4-phenylbutyrate. 30 μM quercetin was added where indicated by the box. Further stimulation by forskolin (10 μM) is also shown. The holding potential was -60 mV.

Figure 7 is a recording illustrating the effect of genistein on G551D-CFTR expressed in a *Xenopus* oocyte. Current was measured with the two-electrode voltage clamp technique. G551D-CFTR was injected in oocyte. Current was first stimulated with forskolin (10 μM) and isobutylmethylxantine (IBMX; 2 mM).

Genistein (50 μM) was added as indicated. The right panel shows current voltage relations recorded after treatment with forskolin and IBMX (F/I) and after treatment with genistein (F/I+Geni). A voltage ramp from -130 mV to +70 mV was applied and current was recorded during the two conditions.

Figure 8 is a recording illustrating the effect of quercetin on nasal potential difference (PD) measurement in a healthy human volunteer. Amiloride (50 μM) was added to block sodium transport as indicated. Conditions were rendered chloride free (Cl free) and chloride secretion was stimulated with isoproterenol (iso; 5 μM). Quercetin (querc; 10 μM) was added as indicated.

Figure 9 is a recording illustrating the effect of apigenin and kaempferol on nasal PD in mice. Chloride secretion was stimulated with isoproterenol (iso; 5 μM), and amiloride (50 μM) was added to block sodium transport as indicated. Under chloride-free conditions (Cl free), apigenin (50 μM, left panel) and kaempferol (kaemp, 50 μM, right panel) were added as indicated.

Figure 10 is a recording illustrating the effect of genistein, with and without 4-phenylbutyrate, on chloride current in JME cells. The recording was performed at 0 mV holding potential with a 17:150 mM chloride gradient from bath to pipette. The bottom trace is from an untreated cell and the top trace is from a cell that had been incubated in 5 mM 4-phenylbutyrate (4-PB) for two days. Forskolin (10 μM) and genistein (30 μM) were added as indicated.

Figures 11A-11C are a whole cell patch clamp recording (Figure 11A) and graphs (Figures 11B and 11C) illustrating the effect of forskolin and genistein on HeLa cells infected with a G551D-CFTR-containing adenovirus. Cells were stimulated with forskolin (10 μM) and genistein (30 μM), as indicated. The fit of the data with the Goldman equation is shown by the line in Figure 11B. A current variance to mean current plot is shown in Figure 11C.

Figure 12A and 12B illustrate the use of representative flavenoids for the treatment of CF patients. Figure 12A shows a recording from a patient with the genotype G551D/ΔF508. Amiloride, chloride free solution and isoproterenol were added as indicated. The addition of genistein, as indicated, hyperpolarized nasal PD.

Figure 12B is a graph illustrating the average responses of nasal PD to genistein and quercetin of four CF patients with the G551D mutation. The filled bars show, for comparison, the respective responses in healthy subjects.

Figures 13A-13C illustrate the effect of additional representative flavenoids and isoflavenoids on chloride current in epithelial cells. Figure 13A is a graph showing the stimulation of transepithelial chloride currents by reservatrol (100 μM), flavanone (100 μM), flavone (200 μM), apigenin (20 μM), apigenin 7-O-neohesperidoside (30 μM), kaempferol (20 μM), fisetin (100 μM), quercetin (30 μM), rutin (30 μM), genistein (30 μM), daidzein (50 μM), biochanin A (100 μM) and prunetin (100 μM) in Calu-3 monolayers. Experiments were performed in the presence of 10 μM forskolin. Stimulated currents are plotted relative to forskolin stimulated increase (forskolin stimulated currents are 100%). Figure 13B is a recording showing the effect of 7,4'-Dihydroxyflavone on chloride current in unstimulated tissue. This recording shows a dose-dependent stimulation of transepithelial short-circuit current (Isc) across Calu-3 monolayers by 7,4'-Dihydroxyflavone. Increasing concentrations of 7,4'-Dihydroxyflavone (as indicated in μM) were added to mucosal side and dose-dependently stimulated chloride currents. Currents were recorded with a serosal-to-mucosal chloride gradient at 0 mV and pulses were obtained at 2 mV. Figure 13C is a recording illustrating the effect of trimethoxy-apigenin. This recording shows dose-dependent stimulation of transepithelial short-circuit current (Isc) across Calu-3 monolayers by trimethoxy-apigenin. Increasing concentrations of trimethoxy-apigenin (as indicated in μM) were added to mucosal side and dose-dependently stimulated chloride currents. Experiment was performed on unstimulated tissue. Currents were recorded with a serosal-to-mucosal chloride gradient at 0 mV and pulses were obtained at 2 mV.

Figure 14 is a recording illustrating the dose-dependent stimulation of transepithelial short-circuit current (Isc) across Calu-3 monolayers by reservatrol. Increasing concentrations of reservatrol (as indicated in μM) were added to the mucosal perfusion and dose-dependently increased chloride currents. For comparison, currents were further stimulated by serosal addition of 20 μM forskolin. Stimulated chloride

current was completely blocked by addition of the chloride channel blocker DPC (5 mM). Currents were recorded with a serosal-to-mucosal chloride gradient at 0 mV and pulses were obtained at 2 mV.

Figure 15 is a recording showing L-ascorbic acid and genistein stimulation of transepithelial short-circuit current (Isc) across Calu-3 monolayers. Ascorbic acid (100 μM) was added as indicated. For comparison, ascorbic acid-stimulated chloride current was subsequently stimulated by the cAMP elevating agonist forskolin (20 μM, serosal). The CFTR activator genistein (20 mM) was then added to the mucosal perfusion as indicated. Stimulated current was completely blocked by addition of the chloride channel blocker DPC (5 mM), added as indicated. Currents were recorded with a serosal-to-mucosal chloride gradient at 0 mV and pulses were obtained at 2 mV.

Figure 16 is a recording showing L-Ascorbic acid and kaempferol stimulation of transepithelial short-circuit current (Isc) across Calu-3 monolayers. 100 μM ascorbic acid and forskolin (fsk, 20 μM, serosal) were added as indicated. The CFTR activator kaempferol (20 μM) was subsequently added, as indicated. Stimulated current was completely blocked by addition of the chloride channel blocker DPC (5 mM). Currents were recorded with a serosal-to-mucosal chloride gradient at 0 mV and pulses were obtained at 2 mV.

Figure 17 is a recording illustrating the effect of L-ascorbic acid on nasal potential difference in human subjects. Amiloride, chloride-free solution and L-ascorbic acid (100 μM) were added to the luminal perfusate in the nose, as indicated. The β-adrenergic agonist isoproterenol was also added as indicated. Stimulation was reversed by washing out drugs with NaCl Ringer solution.

Figure 18 is a recording illustrating the stimulation of transepithelial short-circuit current (Isc) across Calu-3 monolayers by addition of 10, 100 and 300 μM dehydroascorbic acid. Currents were recorded with a serosal-to-mucosal chloride gradient at 0 mV and pulses were obtained at 2mV.

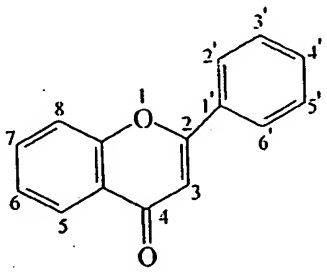
Figure 19 is a recording illustrating the stimulatory effect of 20 μM genistein on transepithelial short-circuit current (Isc) across 31EG4 mammary epithelial

monolayers. Na currents were blocked by mucosal addition of amiloride (10 mM), and chloride currents were further stimulated by forskolin (20 μM, serosal), as indicated. Currents were recorded in symmetrical NaCl Ringers solution at 0 mV and pulses were obtained at 2 mV.

DETAILED DESCRIPTION OF THE INVENTION

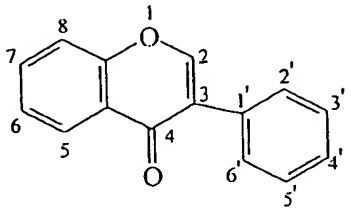
As noted above, the present invention is generally directed to compositions and methods for the treatment of diseases characterized by defective chloride transport in epithelial tissues, including cystic fibrosis, and diseases with excessive accumulation of mucus, including cystic fibrosis, chronic bronchitis and asthma. It has been found, within the context of the present invention, that certain flavones and isoflavones, as well as other polyphenolic compounds, are capable of stimulating CFTR-mediated chloride transport in epithelial tissues (*e.g.*, tissues of the airways, intestine, pancreas and other exocrine glands) in a cyclic-AMP independent manner. Ascorbic acid and derivatives thereof may also, or alternatively, be used within such methods. It has further been found, within the context of the present invention, that such compounds stimulate chloride transport in cells with a mutated CFTR (*e.g.*, ΔF508-CFTR or G551D-CFTR). Such therapeutic compounds may be administered to patients afflicted with cystic fibrosis as described herein.

The term "flavones," as used herein refers to a compound based on the core structure of flavone:



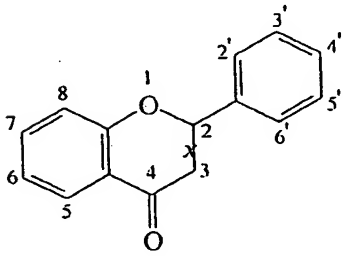
Flavone

An "isoflavone" is an isomer of a flavone (*i.e.*, the phenyl moiety at position 2 is moved to position 3), and having the core structure shown below:

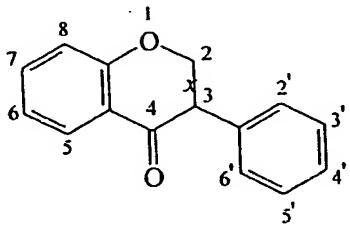


Isoflavone

Certain flavones and isoflavones have the structure:



or



wherein carbon atoms at positions 2, 3, 5, 6, 7, 8, 2', 3', 4', 5' and 6' are bonded to a moiety independently selected from the group consisting of hydrogen atoms, hydroxyl groups and methoxyl groups, and wherein X is a single bond or a double bond. Stereoisomers and glycoside derivatives of such polyphenolic compounds may also be used within the methods provided herein.

Many flavones are naturally-occurring compounds, but synthetic flavones and isoflavones are also encompassed by the present invention. A flavone or isoflavone may be modified to comprise any of a variety of functional groups, such as

hydroxyl and/or ether groups. Preferred flavones comprise one or more hydroxyl groups, such as the trihydroxyflavone apigenin, the tetrahydroxyflavone kaempferol and the pentahydroxyflavone quercetin. Preferred isoflavones comprise one or more hydroxyl and/or methoxy groups, such as the methoxy, dihydroxy isoflavone biochanin A. Genistein is yet another preferred isoflavone for use within the methods provided herein.

Flavones and isoflavones for use within the context of the present invention have the ability to stimulate chloride transport in epithelial tissues. Such transport may result in secretion or absorption of chloride ions. The ability to stimulate chloride transport may be assessed using any of a variety of systems. For example, *in vitro* assays using a mammalian trachea or a cell line, such as the permanent airway cell line Calu-3 (ATCC Accession Number HTB55) may be employed. Alternatively, the ability to stimulate chloride transport may be evaluated within an *in vivo* assay employing a mammalian nasal epithelium. In general, the ability to stimulate chloride transport may be assessed by evaluating CFTR-mediated currents across a membrane by employing standard Ussing chamber (*see* Ussing and Zehrahn, *Acta. Physiol. Scand. 23*:110-127, 1951) or nasal potential difference measurements (*see* Knowles et al., *Hum. Gene Therapy 6*:445-455, 1995). Within such assays, a flavone or isoflavone that stimulates a statistically significant increase in chloride transport at a concentration of about 1 - 300 μM is said to stimulate chloride transport.

Within one *in vitro* assay, the level of chloride transport may be evaluated using mammalian pulmonary cell lines, such as Calu-3 cells, or primary bovine tracheal cultures. In general, such assays employ cell monolayers, which may be prepared by standard cell culture techniques. Within such systems, CFTR-mediated chloride current may be monitored in an Ussing chamber using intact epithelia. Alternatively, chloride transport may be evaluated using epithelial tissue in which the basolateral membrane is permeabilized with *Staphylococcus aureus* α–toxin, and in which a chloride gradient is imposed across the apical membrane (*see* Illek et al., *Am. J. Physiol. 270*:C265-75, 1996). In either system, chloride transport is evaluated in the presence and absence of a test compound (*i.e.*, a flavone or isoflavone), and those

compounds that stimulate chloride transport as described above may be used within the methods provided herein.

Within another *in vitro* assay for evaluating chloride transport, cells are transfected with a chloride channel gene (*e.g.*, CFTR) having a mutation associated with cystic fibrosis. Any CFTR gene that is altered relative to the normal human sequence provided in SEQ ID NO:1, such that the encoded protein contains a mutation associated with cystic fibrosis, may be employed within such an assay. The most common disease-causing mutation in cystic fibrosis is a deletion of phenylalanine at position 508 in the CFTR protein (ΔF508-CFTR; SEQ ID NO:4). Accordingly, the use of a CFTR gene encoding ΔF508-CFTR is preferred. However, genes encoding other altered CFTR proteins (*e.g.*, G551D-CFTR; containing a glycine to aspartate point mutation at position 551; SEQ ID NO:6) may also be used. Cells such as NIH 3T3 fibroblasts may be transfected with an altered CTFR gene, such as ΔF508-CFTR, using well known techniques (*see* Anderson et al., *Science 25*:679-682, 1991). The effect of a compound on chloride transport in such cells may be evaluated by monitoring CFTR-mediated currents using the patch clamp method (*see* Hamill et al., *Pflugers Arch. 391*:85-100, 1981) with and without compound application.

Within another *in vitro* assay, a mutant CFTR may be microinjected into cells such as *Xenopus* oocytes. Chloride conductance mediated by the CFTR mutant in the presence and absence of a test compound may be monitored with the two electrode voltage clamp method (*see* Miledi et al., *Proc. R. Soc. Lond. Biol. 218*:481-484, 1983).

Alternatively, such assays may be performed using a mammalian trachea, such as a primary cow tracheal epithelium using the Ussing chamber technique as described above. Such assays are performed in the presence and absence of test compound to identify flavone and isoflavones that stimulate chloride transport.

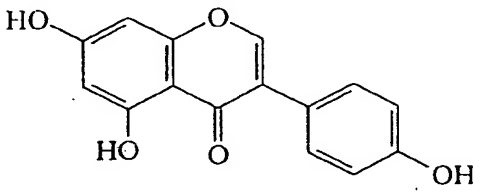
Any of the above assays may be performed following pretreatment of the cells with a substance that increases the concentration of CFTR mutants in the plasma membrane. Such substances include chemical chaperones, which support correct trafficking of the mutant CFTR to the membrane, and compounds that increase expression of CFTR in the cell (*e.g.*, transcriptional activators). A "chemical

chaperone," as used herein is any molecule that increases trafficking of proteins to a cell membrane. More specifically, a chemical chaperone within the context of the present invention increases trafficking of a mutant CFTR (*e.g.*, the Δ508-CFTR and/or G551D-CFTR) to the membrane by a statistically significant amount. Chemical chaperones for use herein include, but are not limited to, glycerol, dimethylsulfoxide, trimethylamine N-oxide, taurin, methylamine and deoxyspergualin (*see* Brown et al., *Cell Stress Chaperones 1*:117-125, 1996; Jiang et al., *Amer J. Physiol.-Cell Physiol. 44*:C171-C178, 1998). Compounds that increase expression of CFTR in the cell include 4-phenylbutyrate (Rubenstein et al., *J. Clin. Invest. 100*:2457-2465, 1997) and sodium butyrate (Cheng et al., *Am. J. Physiol. 268*:L615-624, 1995). Other compounds that increase the level of CFTR in the plasma membrane (by increasing correct trafficking and/or expression of the CFTR) may be readily identified using well known techniques, such as immunohistochemical techniques, to evaluate effects on levels of plasma membrane CFTR.

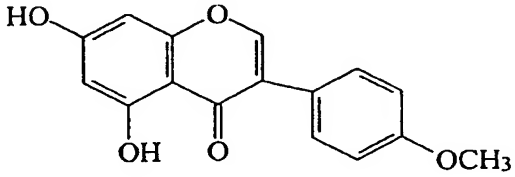
In vivo, chloride secretion may be assessed using measurements of nasal potential differences in a mammal, such as a human or a mouse. Such measurements may be performed on the inferior surface of the inferior turbinate following treatment of the mucosal surface with a test compound during perfusion with the sodium transport blocker amiloride in chloride-free solution. The nasal potential difference is measured as the electrical potential measured on the nasal mucosa with respect to a skin electrode placed on a slightly scratched skin part (*see* Alton et al., *Eur. Respir. J. 3*:922-926, 1990) or with respect to a subcutaneous needle (*see* Knowles et al., *Hum. Gene Therapy 6*:445-455, 1995). Nasal potential difference is evaluated in the presence and absence of test compound, and those compounds that results in a statistically significant increase in nasal potential difference stimulate chloride transport.

Compounds as provided herein may generally be used to chloride transport within any of a variety of CFTR-expressing epithelial cells. CFTR is expressed in may epithelial cells, including intestinal, airway, pancreas, gallbladder, sweat duct, salivary gland and mammary epithelia. All such CFTR-expressing organs are subject to stimulation my the compounds provided herein.

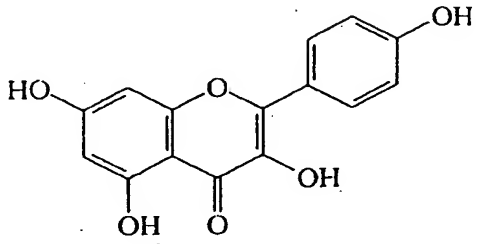
As noted above, any flavone or isoflavone that stimulates chloride transport within at least one of the above assays may be used for therapy of cystic fibrosis, other diseases characterized by abnormally high mucus accumulation in the airways or intestinal disorders such as constipation. Preferred therapeutic compounds include flavones and isoflavones that occur naturally in plants and are part of the human diet. Preferred compounds include genistein (4',5,7-trihydroxyisoflavone), as well as quercetin (3,3',4',5,7-pentahydroxyflavone), apigenin (4'5,7-trihydroxyflavone), kaempferol (3,4',5,7-tetrahydroxyflavone) and biochanin A (4'-methoxy-5,7-dihydroxyisoflavone), as depicted below:



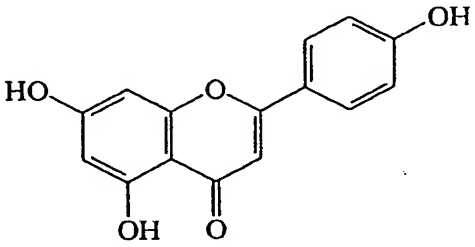
Genistein



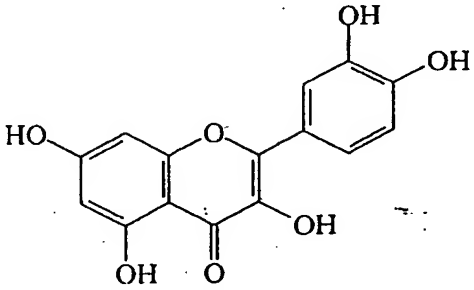
Biochanin A



Kaempferol



Apigenin



Quercetin

Other suitable therapeutic compounds may be identified using the representative assays as described herein. Additional representative flavones and isoflavones include flavanone, flavone, dihydroxyflavone, trimethoxy-apigenin, apigenin 7-O-neohesperidoside, fisetin, rutin, daidzein and prunetin. Representative flavones and isoflavones are summarized in Tables I and II.

Table I

Flavonoids

No.	Name	X	C3	C5	C7	C3'	C4'
1	Apigenin	=		OH	OH		OH
2	Apigenin7-O-neohesperidoside	=		OH	ONeo		OH
3	Dihydroxyflavone	=		OH			OH
4	Flavone	=					
5	Flvanone	-					
6	Fisetin	=	OH		OH	OH	OH
7	Kaempferol	=	OH	OH	OH		OH
8	Quercetin	=	OH	OH	OH	OH	OH
9	Rutin	=	ORut		OH	OH	OH
10	Trimethoxy-apigenin	=	H	OCH3	OCH3		OCH3

where = a double bond, - is a single bond, ONeo is Neohesperidoside, ORut is rutinoside, OCH3 is methoxy, OH is hydroxy

Table II

Isoflavonoids

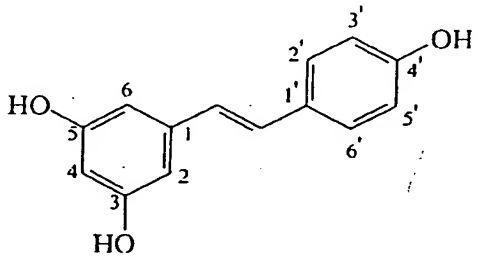
No.	Name	X	C5	C7	C4'
11	Biochanin	=	OH	OH	OCH3
12	Daidzein	=		OH	OH
13	Genistein	=	OH	OH	OH
14	Prunetin	=	OH	OCH3	OH

where = a double bond, - is a single bond, ONeo is Neohesperidoside, ORut is rutinoside, OCH3 is methoxy, OH is hydroxy.

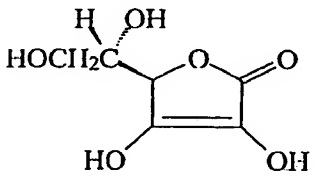
Genistein, quercetin, apigenin, kaempferol, biochanin A and other flavones and isoflavones may generally be prepared using well known techniques, such

as those described by Shakhova et al., *Zh. Obshch. Khim. 32*:390, 1962; Farooq et al., *Arch. Pharm. 292*:792, 1959; and Ichikawa et al., *Org. Prep. Prog. Int. 14*:183, 1981. Alternatively, such compounds may be commercially available (*e.g.*, from Indofine Chemical Co., Inc., Somerville, NJ or Sigma-Aldrich, St. Louis, MO). Further modifications to such compounds may be made using conventional organic chemistry techniques, which are well known to those of ordinary skill in the art.

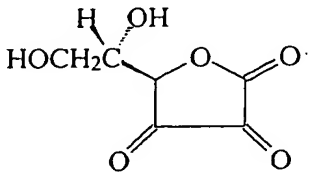
As noted above, other polyphenolic compounds may be used within the methods provided herein. For example, trihydroxystilbenes such as reservatrol (trans-3,5,4'-trihydroxystilbene) may be employed. Reservatrol is a polyphenolic compound having the following structure:



Other compounds that may be used within the methods provided herein are ascorbic acid and derivatives thereof. Such compounds include L-ascorbic acid (L-xyloascorbic acid), dehydroascorbic acid (L-threo-2,3-Hexodiulosonic acid γ-lactone) and salts of the foregoing acids.



L-Ascorbic Acid



Dehydroascorbic Acid

Within certain preferred embodiments, ascorbic acid or a derivative thereof is used in combination with a polyphenolic compound as described above. Certain representative combinations include ascorbic acid and one or more flavenoids and/or isoflavenoids (such as genistein and ascorbic acid; and kaempferol and ascorbic acid). Ascorbic acid may generally be used to treat or prevent genetic loss of chloride secretory function (*e.g.*, cystic fibrosis), as well as other related loss or reduced chloride secretory function (*e.g.*, intestinal constipation, dry eye syndrome and obstructive airway diseases).

For *in vivo* use, a therapeutic compound as described herein is generally incorporated into a pharmaceutical composition prior to administration. Within such compositions, one or more therapeutic compounds as described herein are present as active ingredient(s) (*i.e.*, are present at levels sufficient to provide a statistically significant effect on nasal potential difference, as measured using a representative assay as provided herein). A pharmaceutical composition comprises one or more such compounds in combination with any physiologically acceptable carrier(s) and/or excipient(s) known to those skilled in the art to be suitable for the particular mode of administration. In addition, other pharmaceutically active ingredients (including other therapeutic agents) may, but need not, be present within the composition.

Within certain methods provided herein, a flavone or isoflavone may be combined with a substance that increases the concentration of CFTR mutants in the plasma membrane of a cell. As noted above, such substances include chemical chaperones, which support correct trafficking of the mutant CFTR to the membrane, and compounds that increase expression of CFTR in the membrane. These substances may be contained within the same pharmaceutical composition or may be administered separately. Preferred chemical chaperones include glycerol, dimethylsulfoxide,

trimethylamine N-oxide, taurin, methylamine and deoxyspergualin, and compounds that increase expression of CFTR in the membrane include 4-phenylbutyrate and sodium butyrate. The use of flavenoid and/or isoflavenoid compounds, as described herein, in combination with such substances may increase mutant CFTR activity, and ameliorate symptoms of cystic fibrosis.

Administration may be achieved by a variety of different routes. One preferred route is oral administration of a composition such as a pill, capsule or suspension. Such compositions may be prepared according to any method known in the art, and may comprise any of a variety of inactive ingredients. Suitable excipients for use within such compositions include inert diluents (which may be solid materials, aqueous solutions and/or oils) such as calcium or sodium carbonate, lactose, calcium or sodium phosphate, water, arachis oil, peanut oil liquid paraffin or olive oil; granulating and disintegrating agents such as maize starch, gelatin or acacia and/or lubricating agents such as magnesium stearate, stearic acid or talc. Other inactive ingredients that may, but need not, be present include one or more suspending agents (*e.g.*, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia), thickeners (*e.g.*, beeswax, paraffin or cetyl alcohol), dispersing or wetting agents, preservatives (*e.g.*, antioxidants such as ascorbic acid), coloring agents, sweetening agents and/or flavoring agents.

A pharmaceutical composition may be prepared with carriers that protect active ingredients against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art.

Particularly preferred are methods in which the therapeutic compound(s) are directly administered as a pressurized aerosol or nebulized formulation to the patient's lungs via inhalation. Such formulations may contain any of a variety of known

aerosol propellants useful for endopulmonary and/or intranasal inhalation administration. In addition, water may be present, with or without any of a variety of cosolvents, surfactants, stabilizers (*e.g.*, antioxidants, chelating agents, inert gases and buffers). For compositions to be administered from multiple dose containers, antimicrobial agents are typically added. Such compositions are also generally filtered and sterilized, and may be lyophilized to provide enhanced stability and to improve solubility.

Pharmaceutical compositions are administered in an amount, and with a frequency, that is effective to inhibit or alleviate the symptoms of cystic fibrosis and/or to delay the progression of the disease. The effect of a treatment may be clinically determined by nasal potential difference measurements as described herein. The precise dosage and duration of treatment may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Dosages may also vary with the severity of the disease. A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect while minimizing undesirable side effects. In general, an oral dose ranges from about 200 mg to about 1000 mg, which may be administered 1 to 3 times per day. Compositions administered as an aerosol are generally designed to provide a final concentration of about 10 to 50 μM at the airway surface, and may be administered 1 to 3 times per day. It will be apparent that, for any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

As noted above, a pharmaceutical composition may be administered to a mammal to stimulate chloride transport, and to treat cystic fibrosis. Patients that may benefit from administration of a therapeutic compound as described herein are those afflicted with cystic fibrosis. Such patients may be identified based on standard criteria that are well known in the art, including the presence of abnormally high salt concentrations in the sweat test, the presence of high nasal potentials, or the presence of a cystic fibrosis-associated mutation. Activation of chloride transport may also be beneficial in other diseases that show abnormally high mucus accumulation in the airways, such as asthma and chronic bronchitis. Similarly, intestinal constipation may

benefit from activation of chloride transport by a flavone or isoflavone as provided herein.

Summary of Sequence Listing

SEQ ID NO:1 is a DNA sequence encoding human CFTR.

SEQ ID NO:2 is an amino acid sequence of human CFTR.

SEQ ID NO:3 is a DNA sequence encoding human CFTR with the ΔF508 mutation.

SEQ ID NO:4 is an amino acid sequence of human CFTR with the ΔF508 mutation.

SEQ ID NO:5 is a DNA sequence encoding human CFTR with the G551D mutation.

SEQ ID NO:6 is an amino acid sequence of human CFTR with the G551D mutation.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

Stimulation of Chloride Transport by Representative Flavones and Isoflavones in Airway Cells

This Example illustrates the use of the representative compounds apigenin, quercetin and biochanin A to enhance chloride secretion in Calu-3 human pulmonary cultures or in primary bovine tracheal cultures.

A Calu-3 cell monolayer was prepared in an Ussing chamber as described by Illek et al., *Am. J. Physiol. 270*:C265-275, 1996. The basolateral membrane was permeabilized with α-toxin and a chloride gradient was applied across the apical membrane as a driving force (*see* Illek et al. *Am. J. Physiol. 270*:C265-C275, 1996). The tissue was first stimulated with cAMP (100 μM), and then with a representative flavone or isoflavone.

As shown in Figures 1 and 2, subsequent addition of apigenin or quercetin further stimulated chloride current. Figure 1 illustrates the short circuit current across the Calu-3 cell monolayer before and after addition of apigenin (50 μM). Figure 2 illustrates the effect of quercetin (30 μM) on chloride current across a Calu-3 monolayer. In both cases, the flavone stimulated chloride current beyond the stimulation achieved by cAMP.

Figure 3 illustrates the results of a related experiment to evaluate the dose-dependent stimulation of transepithelial chloride secretion by quercetin across a primary bovine tracheal epithelium. The epithelial cells were first treated with amiloride (50 μM), and then with quercetin at the indicated concentrations. The dose-response relation yielded a half maximal stimulation at 12.5 μM. At high concentrations of quercetin, the current was blocked. Current was fully inhibited by the CFTR channel blocker diphenylcarboxylate (DPC, 5 mM).

To evaluate the effect of biochanin A, a Calu-3 cell monolayer was prepared and permeabilized as described above. The tissue was first stimulated with

forskolin (Fsk, 10 μM). The effect of biochanin A (Bio, 100 and 300 μM) on short-circuit current (I_{sc}) across the Calu-3 monolayer was evaluated in an Ussing chamber. As shown in Figure 4, biochanin A further stimulated chloride secretion.

Example 2

Activation of Mutant CFTR by Representative Flavones and Isoflavones

This Example illustrates the use of the representative compounds apigenin, quercetin and genistein to activate ΔF508-CFTR and G551D-CFTR in different cell types.

A cell-attached single channel patch clamp recording was obtained from a 3T3 cell expressing ΔF508-CFTR as described by Hamill et al., *Pflugers Arch. 391*:85-100, 1981 and Fischer and Machen, *J. Gen. Physiol. 104*:541-566, 1994. As shown in Figure 5, stimulation of the cell with 10 μM forskolin did not activate ΔF508-CFTR channel, but addition of genistein (50 μM) or apigenin (50 μM, where indicated by boxes) induced ΔF508-CFTR channel openings, and removal of these compounds inactivated the channels. The holding potential was 75 mV, and channel openings were upward.

Figure 6 presents a whole cell patch clamp recording on an airway epithelial cell homozygous for ΔF508-CFTR (cell type: JME cell, *see* Jeffersen et al., *Am. J. Physiol. 259*:L496-L505, 1990). Before the measurement, the cell was incubated for 2 days in 5 mM 4-phenylbutyrate to enhance ΔF508-CFTR expression in the plasma membrane (Rubenstein & Zeitlin, *Ped. Pulm. Suppl. 12*:234, 1995). Measurements were performed as described by Fischer et al., *J. Physiol. Lond. 489*:745-754, 1995. Addition of 30 μM quercetin activated chloride current in the whole cell mode, which was further stimulated by forskolin. The holding potential was -60 mV.

The effect of genistein on chloride current in a *Xenopus* oocyte expressing G551D-CFTR was measured with the two-electrode voltage clamp technique (*see* Miledi et al., *Proc. R. Soc. Lond. Biol. 218*:481-484, 1983). G551D-

CFTR (2 ng in 50 nL of water) was injected into the oocyte. Current was first stimulated with forskolin (10 μM) and isobutylmethylxantine (IBMX; 2 mM). Genistein (50 μM) was found to further activate chloride currents. As shown in Figure 7, genistein increased conductance and shifted reversal potential to the right, which is indicative of a stimulated chloride current.

Example 3

Effect of Representative Flavones on Nasal Potential Difference

This Example illustrates the *in vivo* use of quercetin, apigenin and kaempferol to activate the nasal potential difference in humans and mice.

The effect of quercetin on nasal potential difference (PD) measurement in a healthy human volunteer was measured as described by Knowles et al., *Hum. Gene Therapy 6*:445-455, 1995. Under conditions where sodium transport was blocked with amiloride (50 μM) and chloride secretion was stimulated under chloride-free conditions with isoproterenol (5 μM), quercetin (10 μM) stimulated nasal PD further (Figure 8).

The effect of apigenin and kaempferol on nasal PD in mice was evaluated using a method similar to that employed for measurements in humans, except that a plastic tube of approximately 0.1 mm diameter was used as an exploring nasal electrode. The plastic tube was perfused with test solutions at approximately 10 μL/min. After blocking sodium transport with amiloride (50 μM) and during stimulation of chloride secretion with isoproterenol (iso;5 μM) under chloride-free conditions, apigenin (50 μM, left panel) and kaempferol (kaemp. 50 μM, right panel) further stimulated nasal PD.

These results show that the representative flavenoids quercetin, apigenin, kaempferol and biochanin A stimulate chloride transport across epithelial tissues derived from the airways *in vitro*, and across nasal epithelium *in vivo*. The results also show that the CFTR mutants ΔF508 and G551D can be activated by the representative compounds genistein and apigenin.

Example 4

Effect of Genistein on Chloride Current in Cells Expressing a Mutant CFTR

This Example illustrates the ability of the representative isoflavone genistein to activate chloride current in cells expressing a mutant CFTR.

In one experiment, genistein was used in combination with 4-phenylbutyrate. Chloride current was measured in JME cells (human nasal epithelial cell line homozygous for the Δ508 mutation of CFTR; *see* Jefferson et al., *Am. J. Physiol. 259*:L496-505, 1990). The recording was performed at 0 mV holding potential with a 17:150 mM chloride gradient from bath to pipette. Under these conditions, the recorded current, shown in Figure 10, is chloride current (Illek and Fischer, *Am. J. Physiol.* (*Lung Cell. Mol. Physiol.*):L902-910, 1998). The bottom trace in Figure 10 is from an untreated cell. Neither forskolin (10 μM nor genistein (30 μM activated current. The top tracing in Figure 10 is from a cell that had been incubated in 5 mM 4-phenylbutyrate (4-PB) for two days (Rubenstein et al., *J. Clin. Invest. 100*:2457-2465, 1997). After 4-PB treatment, chloride current was stimulated by forskolin (by on average 30.3 ± 19.4 pS/pF, n=6), and further activated by genistein (to an average 105 ± 84 pS/pF) in a CF cell with the Δ508-CFTR mutation. These results further demonstrate the ability of a flavenoid compound to optimize chloride currents elicited in CF cells by other means.

Within another experiment, HeLa cells infected with the G551D-CFTR-containing adenovirus were investigated in the patch clamp mode. Stimulation of the cell with forskolin (10 μM) stimulated only a very small current (Figures 11A and 11B). On average, forskolin-stimulated conductance was 9.5 ± 5 pS/pF (n=4). Additional stimulation with genistein (30 μM) stimulated significant chloride currents, which were time- and voltage-independent (Figure 11B) and well fitted with the Goldman equation (line in Figure 11B; Illek and Fischer, *Am. J. Physiol.* (*Lung Cell. Mol. Physiol.*):L902-910, 1998), which are characteristics of CFTR-mediated currents. Average forskolin + genistein-activated conductance was 120 ± 30 pS/pF (n=4). Current variance to mean current plot (Figure 11C) were used to calculate the average

open probability (P_o shown on top of axis) of the population of channels carrying the total current (as described in Illek and Fischer, *Am. J. Physiol.* (*Lung Cell. Mol. Physiol.*):L902-910, 1998). During forskolin stimulation, maximal P_o reached was 0.04 (open circles) and after additional stimulation with genistein P_o reached a maximum of 0.42 in this recording. On average, after forskolin stimulation, $P_o = 0.05 \pm 0.02$ and after forskolin + genistein stimulation $P_o = 0.54 \pm 0.12$. For comparison, wild type CFTR expressed in HeLa cells and recorded under the same conditions resulted in $P_o = 0.36 \pm 0.05$ (n=3) after forskolin stimulation and $P_o = 0.63 \pm 0.16$ after forskolin + genistein treatment.

Example 5

Effect of Representative Flavones on Nasal Potential Difference in CF Patients

This Example illustrates the *in vivo* use of quercetin and genistein to activate the nasal potential difference in CF patients bearing the G551D mutation.

Measurements were performed on patients as described by Alton et al., *Eur. Respir. J.* 3:922-926, 1990; Illek and Fischer, *Am. J. Physiol.* (*Lung Cell. Mol. Physiol.*):L902-910, 1998; and Knowles et al., *Hum. Gene Therapy* 6:445-455, 1995). The results are presented in Figures 12A and 12B. Figure 12A shows a recording from a patient with the genotype G551D/ΔF508. Initial treatment with amiloride and chloride free solution had the purpose to isolate and amplify the chloride selective potentials. Addition of the beta-adrenergic agonist isoproterenol showed no effect, which is typical for CF patients (Knowles et al., *Hum. Gene Therapy* 6:445-455, 1995). However, addition of genistein hyperpolarized nasal PD. Average responses of nasal PD to genistein and quercetin of four CF patients with the G551D mutation are shown in Figure 12B (open bars). The filled bars show for comparison the respective responses in healthy subjects. The genotypes of the 4 CF patients were: two G551D/ΔF508, one G551D/G551D and one G551D/unknown. Responses are most likely due to the G551D mutation because the homozygous G551D patient responded

not different compared to the heterozygous G551D patients. Genistein and quercetin responses of nasal PD in CF patients were significant ($p<0.05$).

These results demonstrate that CFTR mutations are sensitive to flavenoid treatment, and provide additional evidence for therapeutic benefit of such compounds for the treatment of cystic fibrosis.

Example 6

Effect of Additional Representative Polyphenolic Compounds on Epithelial Cell Chloride Currents

This Example illustrates the effect of further flavenoids and isoflavenoids on chloride currents in airway epithelial cells.

Airway epithelial cells were prestimulated with 10 μM forskolin. The percent increase in chloride current was then determined following treatment with a series of polyphenolic compounds. Figure 13A summarizes the stimulatory effect of these compounds. On average, chloride currents were further stimulated by reservatrol (100 μM) to 135%, by flavanone (100 μM) to 140%, by flavone (200 μM) to 128%, by apigenin (20 μM) to 241%, by apigenin 7-O-neohesperidoside (30 μM) to 155%, by kaempferol (20 μM) to 182%, by fisetin (100 μM) to 108%, by quercetin (30 μM) to 169%, by rutin (30 μM) to 149%, by genistein (30 μM) to 229%, by daidzein (50 μM) to 162%, by biochanin A (100 μM) to 139% and by prunetin (100 μM) to 161%.

The stimulatory effect of 7,4' Dihydroxyflavone is shown in Figure 13B. Addition of 7,4'-Dihydroxyflavone to the mucosal perfusion dose-dependently stimulated transepithelial Cl currents in unstimulated Calu-3 monolayers. This experiment was performed using unstimulated tissue.

The stimulatory effect of trimethoxy-apigenin is shown in Figure 13C. Addition of trimethoxy-apigenin to the mucosal perfusion dose-dependently stimulated transepithelial Cl currents in unstimulated Calu-3 monolayers. Kinetic analysis is depicted on the right panel and estimated half maximal stimulatory dose was 11.7 μM.

These results indicate that a variety of polyphenolic compounds stimulate chloride currents in epithelial cells.

Example 7

Effect of Reservatrol on Chloride Currents

This Example illustrates the stimulatory effect of reservatrol on transepithelial chloride currents.

Unstimulated Calu-3 monolayers were treated with increasing concentrations of reservatrol. Figure 14 shows the recording generated following the addition of reservatrol to the mucosal perfusion dose-dependently stimulated transepithelial chloride currents in unstimulated Calu-3 monolayers. For comparison, currents were further stimulated by serosal addition of forskolin. The stimulated chloride current was completely blocked by the C1 channel blocker DPC. These results indicate that reservatrol stimulates transepithelial chloride transport.

Example 8

Effect of Ascorbic Acid and Dehydroascorbic Acid on Chloride Currents

This Example illustrates the stimulatory effect of ascorbic acid and dehydroascorbic acid on transepithelial chloride current.

Unstimulated Calu-3 monolayers were stimulated with L-ascorbic acid, as shown in Figure 15. Addition of L-ascorbic acid to the mucosal or serosal perfusion very effectively stimulated transepithelial chloride secretion in unstimulated Calu-3 monolayers. For comparison, chloride currents were further stimulated by serosal addition of forskolin. In the continued presence of L-ascorbic acid and forskolin, it is remarkable that addition of genistein further stimulated chloride currents. These results indicate that genistein serves as a potent drug that is able to hyperstimulate chloride secretion and thereby maximize chloride transport across epithelia. The stimulated chloride current was completely blocked by the chloride channel blocker DPC.

The stimulatory effect of L-ascorbic acid is also shown in Figure 16. Addition of 100 μM L-ascorbic acid to the mucosal or serosal perfusion very effectively stimulated transepithelial chloride currents in unstimulated Calu-3 monolayers. For comparison, ascorbic acid-stimulated chloride currents were stimulated by the cAMP elevating agonist forskolin (20 μM, serosal). Under these stimulated conditions kaempferol further hyperstimulated chloride currents. The stimulated chloride current was completely blocked by the chloride channel blocker DPC (5 mM).

The stimulatory effect of dehydroascorbic acid is shown in Figure 18. Addition of dehydroascorbic acid at 10, 100 or 300 μM to the mucosal and serosal perfusion effectively stimulated transepithelial chloride currents in unstimulated Calu-3 monolayers. Stimulated Cl currents returned to baseline after 5-15 min.

Example 9

Effect of Ascorbic Acid on Chloride Currents *in vivo*

This Example illustrates the stimulatory effect of ascorbic acid on human nasal potential difference.

Nasal potential difference measurement was performed on a human volunteer according to a protocol by Knowles et al., *Hum. Gene Therapy* 6:445-455, 1995. Addition of L-ascorbic acid (100 μM) to the luminal perfusate in the nose (in the presence of amiloride (blocks Na currents) and in chloride-free solution) hyperpolarized nasal potential difference (PD) by 6.3 mV (Figure 17). Addition of the β-adrenergic agonist isoproterenol further hyperpolarized nasal PD. Stimulation was reversed by washing out drugs with NaCl Ringer solution. These results demonstrate the ability of ascorbic acid to stimulate chloride transport in epithelia in humans.

Example 10

Effect of Genistein on Chloride Currents in Mammary Epithelia

This Example illustrates the stimulatory effect of genistein in mammary epithelial cells.

The stimulation of transepithelial short-circuit current (Isc) across 31EG4 mammary epithelial monolayers by addition of 20 μM genistein is shown in Figure 19. Na currents were blocked by mucosal addition of amiloride (10 mM). Chloride currents were further stimulated by forskolin (20 μM, serosal). Currents were recorded in symmetrical NaCl Ringers solution at 0 mV and pulses were obtained at 2 mV.

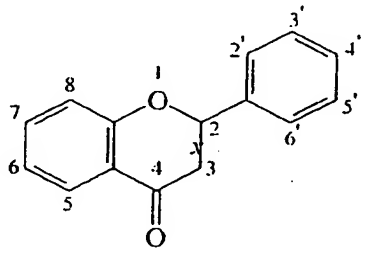
From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

Claims

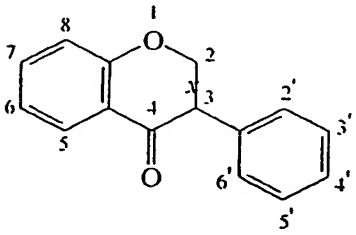
1. A method for enhancing chloride transport in epithelial cells, comprising contacting epithelial cells with a compound selected from the group consisting of flavones and isoflavones, wherein the compound is capable of stimulating chloride transport, and wherein the compound is not genistein.

2. A method according to claim 1, wherein the compound is:

(a) a polyphenolic compound having the general formula:



or



wherein carbon atoms at positions 2, 3, 5, 6, 7, 8, 2', 3', 4', 5' and 6' are bonded to a moiety independently selected from the group consisting of hydrogen atoms, hydroxyl groups and methoxyl groups, and wherein X is a single bond or a double bond; or

(b) a stereoisomer or glycoside derivative of any of the foregoing polyphenolic compounds.

3. A method according to claim 1, wherein the compound is selected from the group consisting of quercetin, apigenin, kaempferol, biochanin A, flavanone, flavone,

dihydroxyflavone, trimethoxy-apigenin, apigenin 7-O-neohesperidoside, fisetin, rutin, daidzein and prunetin.

4. A method for enhancing chloride transport in epithelial cells, comprising contacting epithelial cells with a compound selected from the group consisting of reservatrol, ascorbic acid, ascorbate salts and dehydroascorbic acid.

5. A method according to claim 1 or claim 4, wherein the epithelial cells are airway epithelial cells.

6. A method according to claim 5, wherein the airway epithelial cells are present in a mammal.

7. A method according to claim 6, wherein the compound is administered orally.

8. A method according to claim 6, wherein the compound is administered by inhalation.

9. A method according to claim 1 or claim 4, wherein the epithelial cells are intestinal cells.

10. A method according to claim 9, wherein the intestinal epithelial cells are present in a mammal.

11. A method according to claim 10, wherein the compound is administered orally.

12. A method according to claim 1 or claim 4, wherein the epithelial cells are pancreas, gallbladder, sweat duct, salivary gland or mammary epithelial cells.

13. A method according to claim 12, wherein the intestinal epithelial cells are present in a mammal.

14. A method according to claim 1 or claim 4, wherein the cells are further contacted with a substance that increases (a) trafficking of a CFTR to the plasma membrane of the cells: and/or (b) expression of a CFTR in the cells.

15. A method according to claim 1 or claim 4, wherein the compound is present within a pharmaceutical composition comprising a physiologically acceptable carrier or excipient.

16. A method according to claim 1 or claim 4, wherein the epithelial cells produce a mutated CFTR protein.

17. A method according to claim 16, wherein the mutated CFTR protein has a deletion at position 508 or a point mutation at position 551.

18. A method according to claim 1 or claim 4, wherein the pharmaceutical composition further comprises a substance that increases (a) trafficking of a CFTR to the plasma membrane of the cells: and/or (b) expression of a CFTR in the cells.

19. A method according to claim 18, wherein the substance increases expression of a CFTR in the cells and is 4-phenylbutyrate or sodium butyrate.

20. A method according to claim 18, wherein the substance is a chemical chaperone that increases trafficking of a CFTR to the plasma membrane of the cells, and wherein the compound is selected from the group consisting of glycerol, dimethylsulfoxide, trimethylamine N-oxide, taurin, methylamine and deoxyspergualin.

21. A method according to claim 1, wherein the cells are further contacted with a compound selected from the group consisting of reservatrol, ascorbic acid, ascorbate salts and dehydroascorbic acid.

22. A method according to claim 21, wherein the cells are contacted with a polyphenolic compound and ascorbic acid.

23. A method according to claim 22, wherein the polyphenolic compound is genistein, daidzein or prunetin.

24. A method for enhancing chloride transport in epithelial cells, comprising contacting epithelial cells with genistein, wherein the epithelial cells produce a mutated CFTR protein.

25. A method according to claim 24, wherein the mutated CFTR protein is G551D-CFTR or ΔF508-CFTR.

26. A method according to claim 24, wherein the epithelial cells are further contacted with a substance that increases (a) trafficking of a CFTR to the plasma membrane of the cells; and/or (b) expression of a CFTR in the cells.

27. A method according to claim 26, wherein the substance increases expression of a CFTR in the cells and is 4-phenylbutyrate or sodium butyrate.

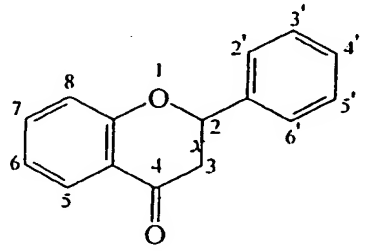
28. A method according to claim 26, wherein the substance is a chemical chaperone that increases trafficking of a CFTR to the plasma membrane of the cells, and wherein the compound is selected from the group consisting of glycerol, dimethylsulfoxide, trimethylamine N-oxide, taurin, methylamine and deoxyspergualin.

29. A method for enhancing chloride transport in epithelial cells, comprising contacting epithelial cells with genistein and a compound selected from the group consisting of reservatrol, ascorbic acid, ascorbate salts and dehydroascorbic acid.

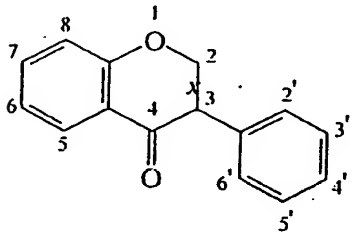
30. A method for treating cystic fibrosis in a mammal, comprising administering to a mammal one or more compounds selected from the group consisting of flavones and isoflavones, wherein the compound is capable of stimulating chloride secretion, and thereby treating cystic fibrosis in the mammal.

31. A method according to claim 30, wherein the compound is:

(a) a polyphenolic compound having the general formula:



or



wherein carbon atoms at positions 2, 3, 5, 6, 7, 8, 2', 3', 4', 5' and 6' are bonded to a moiety independently selected from the group consisting of hydrogen atoms, hydroxyl groups and methoxyl groups, and wherein X is a single bond or a double bond; or

(b) a stereoisomer or glycoside derivative of any of the foregoing polyphenolic compounds.

32. A method according to claim 31, wherein the compound is selected from the group consisting of quercetin, apigenin, kaempferol, biochanin A, flavanone,

flavone, dihydroxyflavone, trimethoxy-apigenin, apigenin 7-O-neohesperidoside, fisetin, rutin, daidzein and prunetin.

33. A method according to claim 31, wherein the compound is genistein.

34. A method for treating cystic fibrosis in a mammal, comprising administering to a mammal one or more compounds selected from the group consisting of reservatrol, ascorbic acid, ascorbate salts and dehydroascorbic acid.

35. A method according to claim 30 or claim 34, wherein the compound is administered orally.

36. A method according to claim 30 or claim 34, wherein the compound is administered by inhalation.

37. A method according to claim 30 or claim 34, wherein a substance is further administered to the mammal, such that the substance increases (a) trafficking of a CFTR to the plasma membrane of epithelial cells; and/or (b) expression of a CFTR in epithelial cells.

38. A method according to claim 37, wherein the substance increases expression of a CFTR in the cells and is 4-phenylbutyrate or sodium butyrate.

39. A method according to claim 37, wherein the substance is a chemical chaperone that increases trafficking of a CFTR to the plasma membrane of the cells, and wherein the compound is selected from the group consisting of glycerol, dimethylsulfoxide, trimethylamine N-oxide, taurin, methylamine and deoxyspergualin.

40. A method according to claim 30, wherein the compound is present within a pharmaceutical composition comprising a physiologically acceptable carrier or excipient.

41. A method according to claim 40, wherein the pharmaceutical composition further comprises a compound selected from the group consisting of resevatrol, ascorbic acid, ascorbate salts and dehydroascorbic acid.

42. A method according to claim 40, wherein the pharmaceutical composition further comprises a substance that increases (a) trafficking of a CFTR to the plasma membrane of epithelial cells; and/or (b) expression of a CFTR in epithelial cells.

43. A method according to claim 40, wherein the substance increases expression of a CFTR in the cells and is 4-phenylbutyrate or sodium butyrate.

44. A method according to claim 42, wherein the substance is a chemical chaperone that increases trafficking of a CFTR to the plasma membrane of the cells, and wherein the compound is selected from the group consisting of glycerol, dimethylsulfoxide, trimethylamine N-oxide, taurin, methylamine and deoxyspergualin.

45. A method for increasing chloride ion conductance in airway epithelial cells of a patient afflicted with cystic fibrosis, wherein the patient's CFTR protein has a deletion at position 508, the method comprising administering to a mammal one or more compounds selected from the group consisting of flavones and isoflavones, wherein the compound is capable of stimulating chloride secretion.

46. A method according to claim 45, wherein the compound is genistein.

47. A method according to claim 45, wherein the compound is quercetin.

48. A method for increasing chloride ion conductance in airway epithelial cells of a patient afflicted with cystic fibrosis, wherein the patient's CFTR protein has a mutation at position 551, the method comprising administering to a mammal one or more compounds selected from the group consisting of flavones and isoflavones, wherein the compound is capable of stimulating chloride secretion.

49. A method according to claim 48, wherein the compound is genistein.

50. A method according to claim 48, wherein the compound is quercetin.

51. A pharmaceutical composition for treatment of cystic fibrosis, comprising:

(a) one or more flavones or isoflavones capable of stimulating chloride secretion;

(b) one or more of:

(i) a compound that increases expression of a CFTR in an epithelial cell; and/or

(ii) a chemical chaperone that increases trafficking of a CFTR to a plasma membrane in an epithelial cell; and

(c) a physiologically acceptable carrier.

52. A pharmaceutical composition for treatment of cystic fibrosis, comprising:

(a) genistein;

(b) one or more of:

(i) a compound that increases expression of a CFTR in an epithelial cell; and/or

(ii) a chemical chaperone that increases trafficking of a CFTR to a plasma membrane in an epithelial cell; and

(c) a physiologically acceptable carrier.

53. A pharmaceutical composition for treatment of cystic fibrosis, comprising:

(a) quercetin;

(b) one or more of:

(i) a compound that increases expression of a CFTR in an epithelial cell; and/or

(ii) a chemical chaperone that increases trafficking of a CFTR to a plasma membrane in an epithelial cell; and

(c) a physiologically acceptable carrier.

54. A pharmaceutical composition for treatment of cystic fibrosis, comprising:

(a) apigenin;

(b) one or more of:

(i) a compound that increases expression of a CFTR in an epithelial cell; and/or

(ii) a chemical chaperone that increases trafficking of a CFTR to a plasma membrane in an epithelial cell; and

(c) a physiologically acceptable carrier.

55. A pharmaceutical composition for treatment of cystic fibrosis, comprising:

(a) kaempferol;

(b) one or more of:

(i) a compound that increases expression of a CFTR in an epithelial cell; and/or

(ii) a chemical chaperone that increases trafficking of a CFTR to a plasma membrane in an epithelial cell; and

(c) a physiologically acceptable carrier.

56. A pharmaceutical composition for treatment of cystic fibrosis, comprising:

(a) biochanin A;

(b) one or more of:

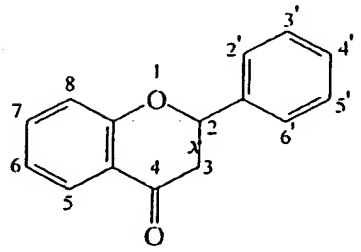
(i) a compound that increases expression of a CFTR in an epithelial cell; and/or

(ii) a chemical chaperone that increases trafficking of a CFTR to a plasma membrane in an epithelial cell; and

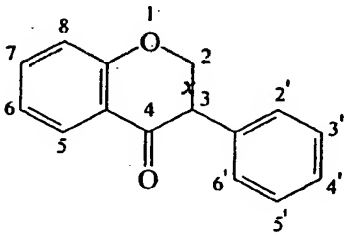
(c) a physiologically acceptable carrier.

57. A pharmaceutical composition for treatment of cystic fibrosis, comprising:

(a) a polyphenolic compound having the general formula:



or



wherein carbon atoms at positions 2, 3, 5, 6, 7, 8, 2', 3', 4', 5' and 6' are bonded to a moiety independently selected from the group consisting of hydrogen atoms, hydroxyl groups and methoxyl groups, and wherein X is a single bond or a double bond; or a stereoisomer or glycoside derivative of any of the foregoing polyphenolic compounds;

(b) a compound selected from the group consisting of reservatrol, ascorbic acid, ascorbate salts and dehydroascorbic acid; and

(c) a physiologically acceptable carrier.

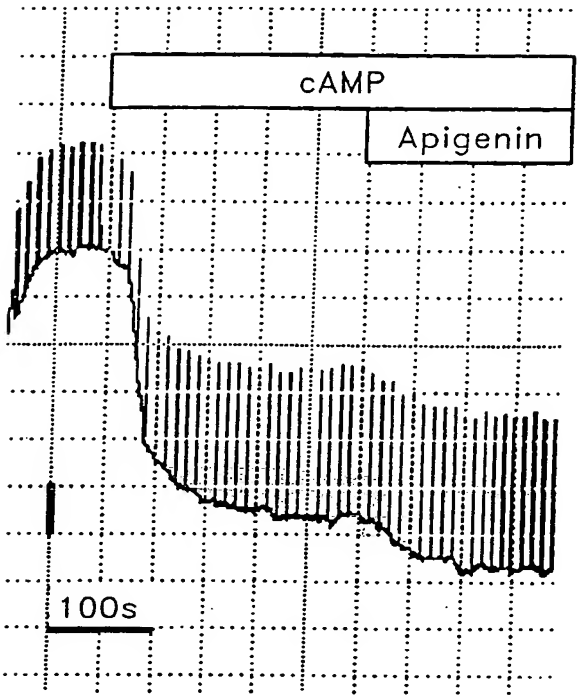


Fig. 1

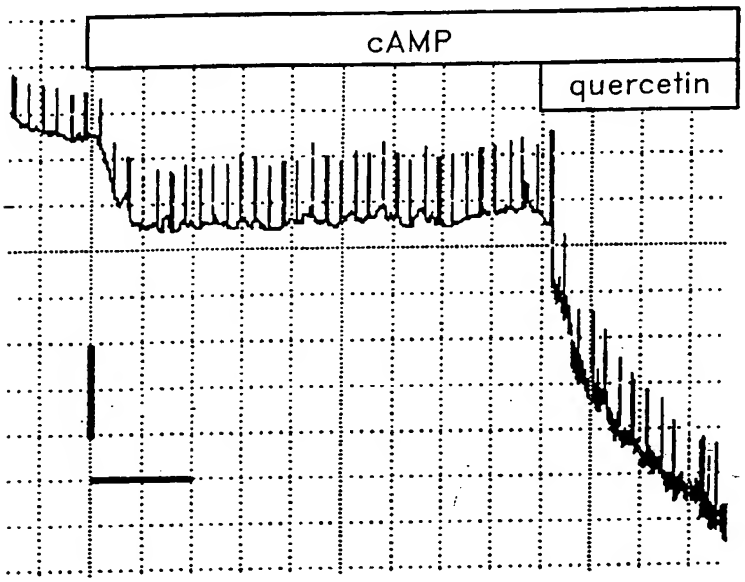


Fig. 2

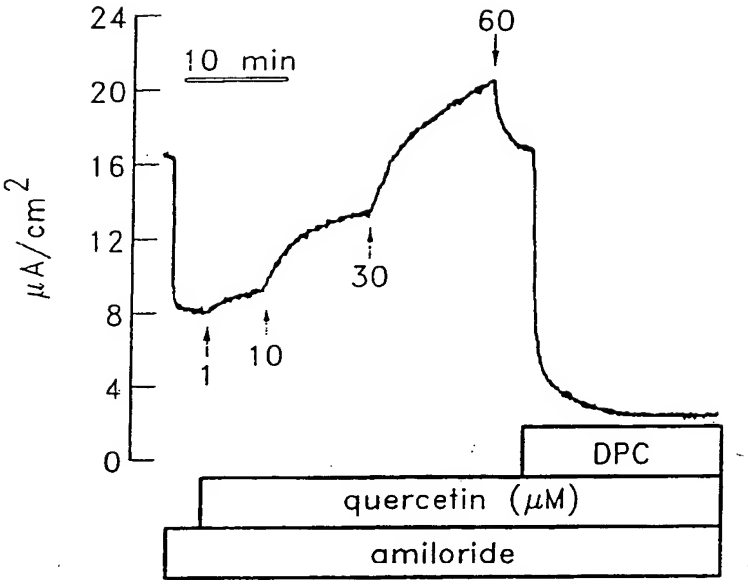

24
20
16
12
8
4
0
μA/cm2
10 min
60
30
10
1
DPC
quercetin (μM)
amiloride

Fig. 3

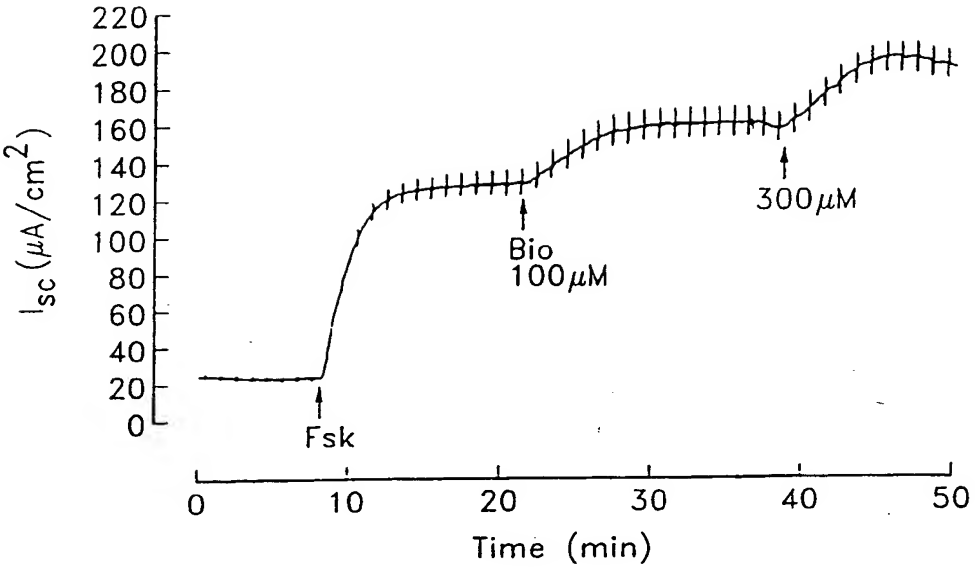


Fig. 4

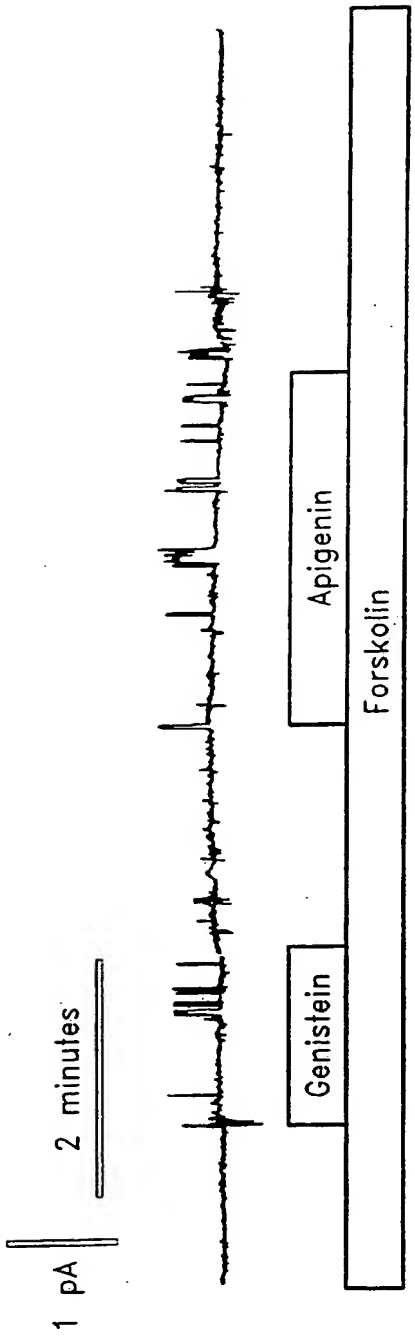


Fig. 5

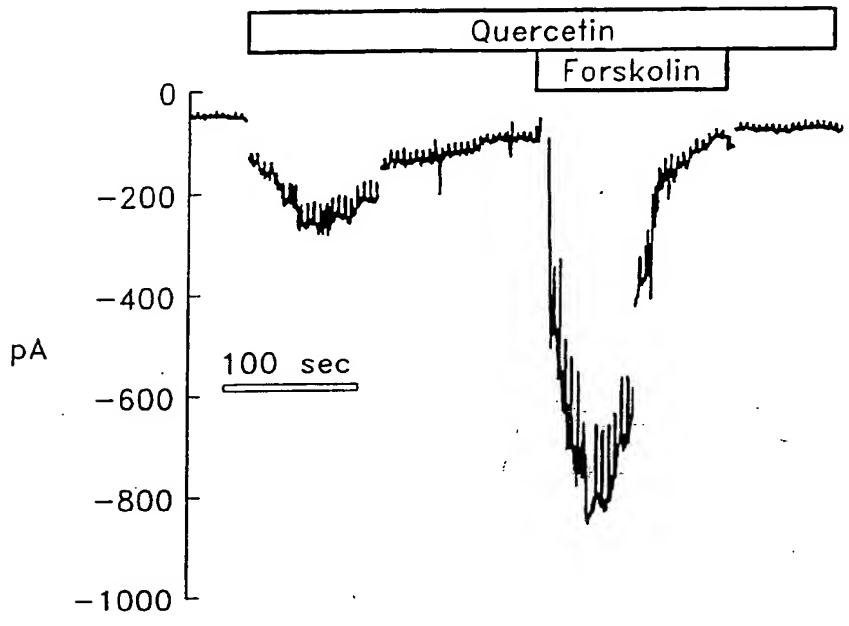


Fig. 6

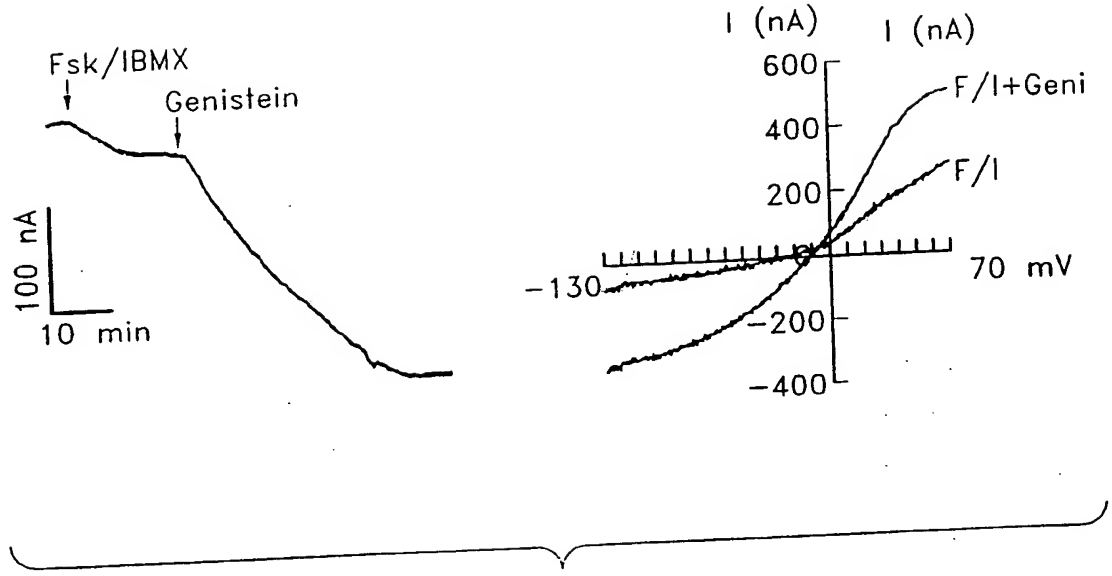


Fig. 7

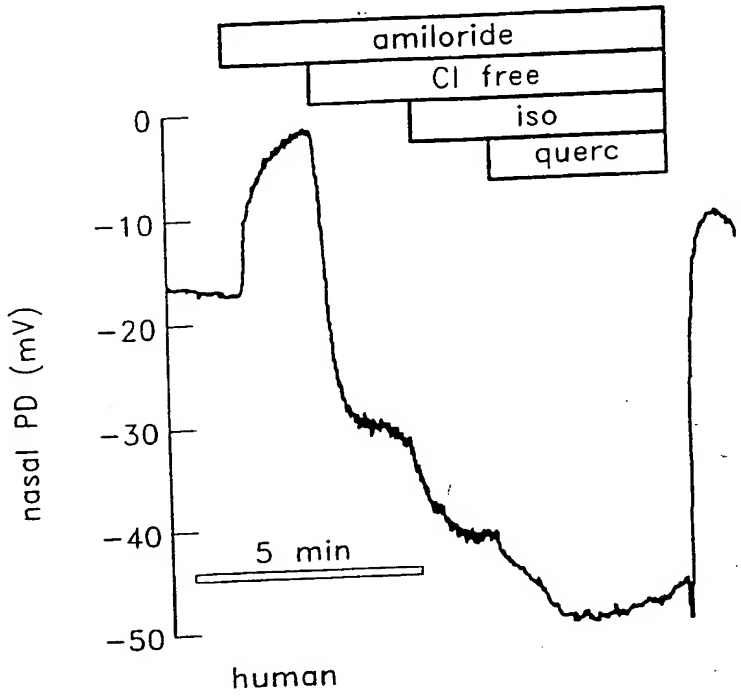

amiloride
Cl free
iso
querc
0
−10
−20
−30
−40
−50
nasal PD (mV)
5 min
human

Fig. 8

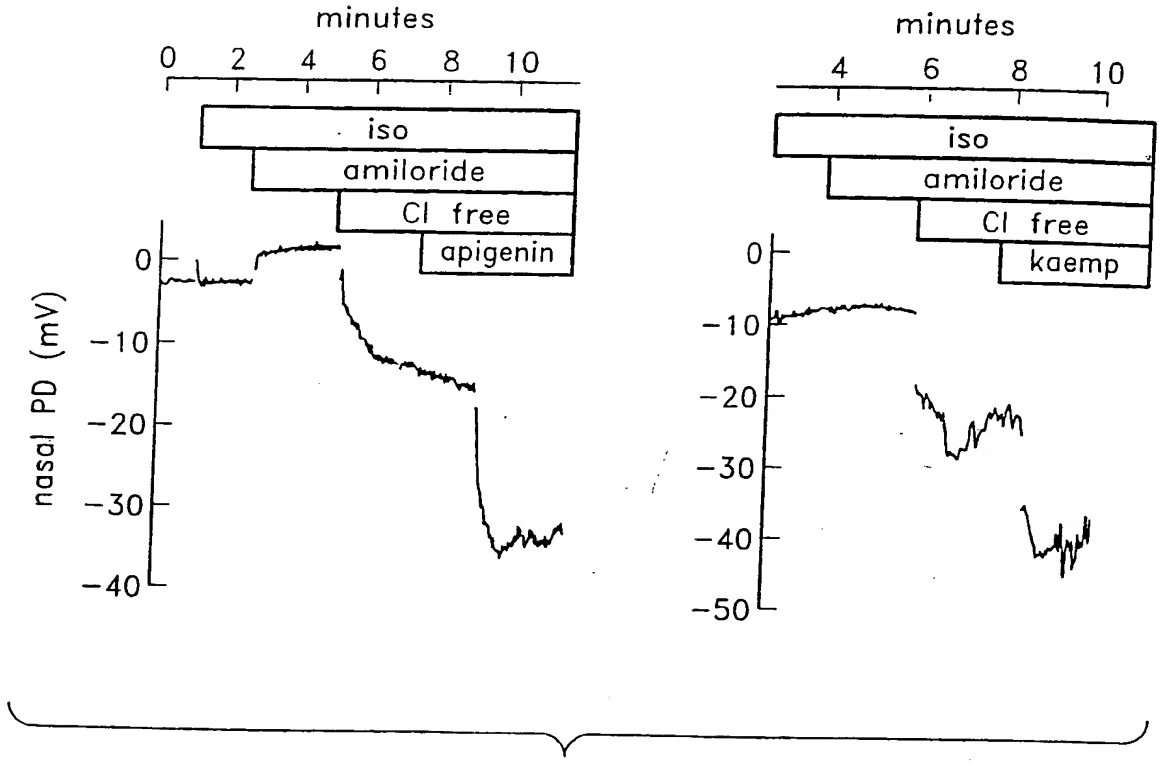


Fig. 9

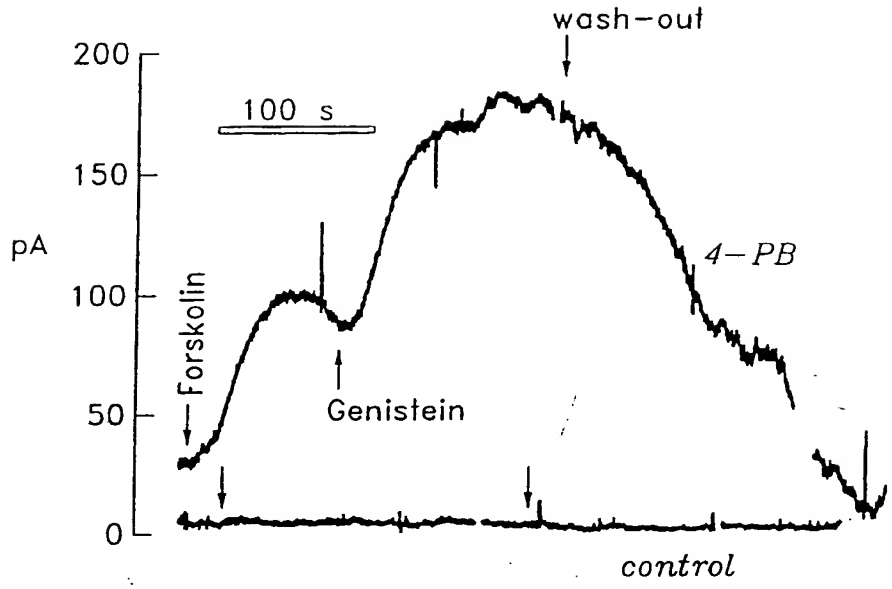

wash-out
200
100 s
150
pA
100
4-PB
Forskolin
Genistein
50
0
control

Fig. 10

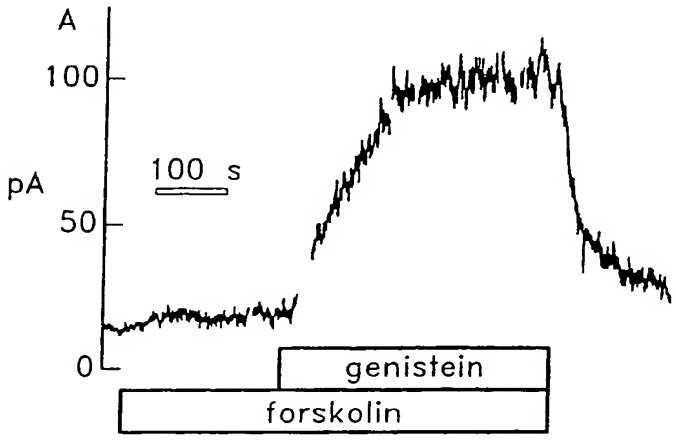


Fig. 11A

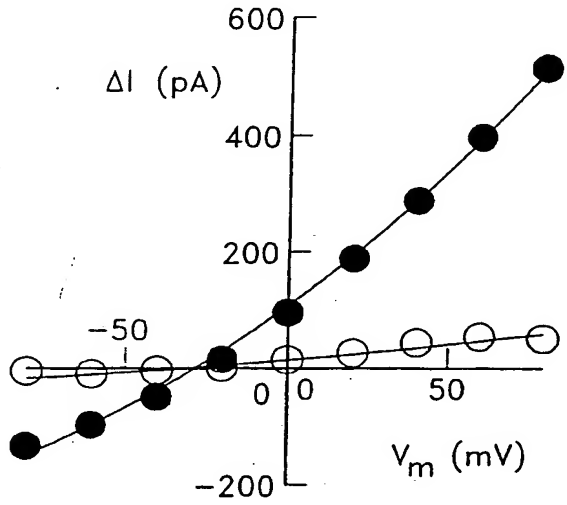


Fig. 11B

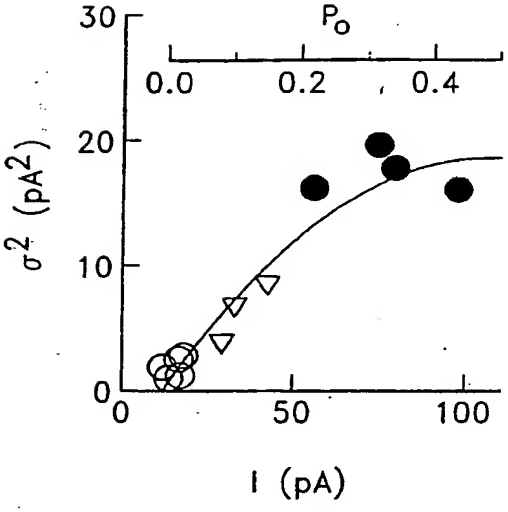


Fig. 11C

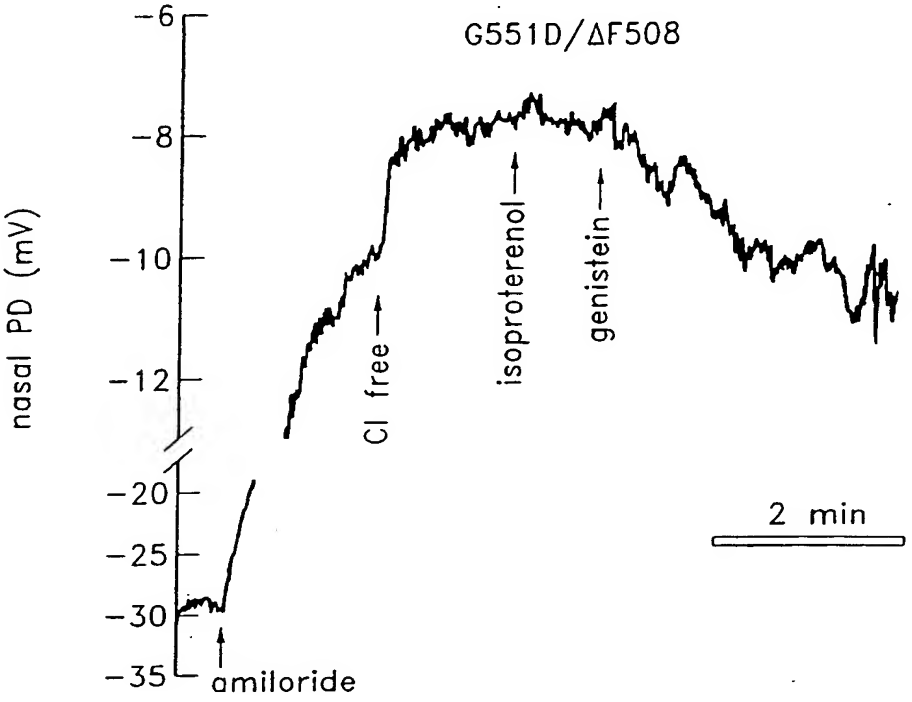


Fig. 12A

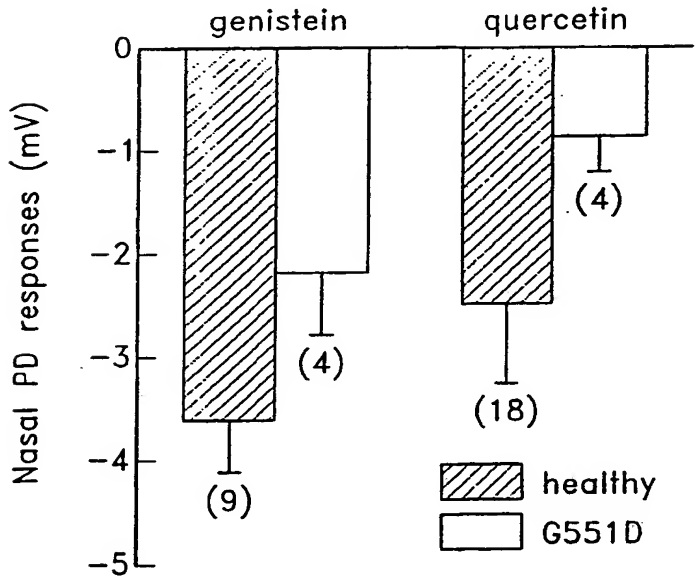


Fig. 12B

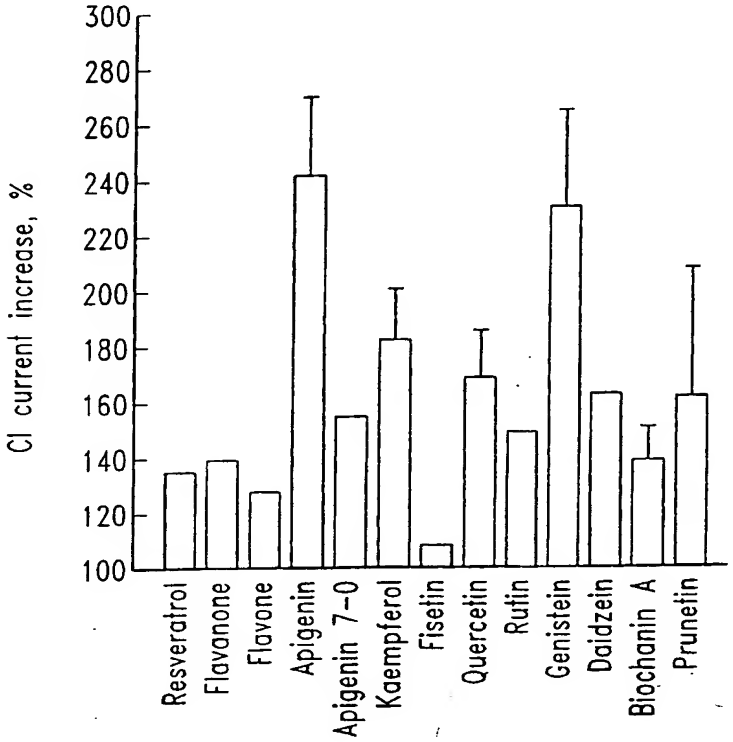


Fig. 13A

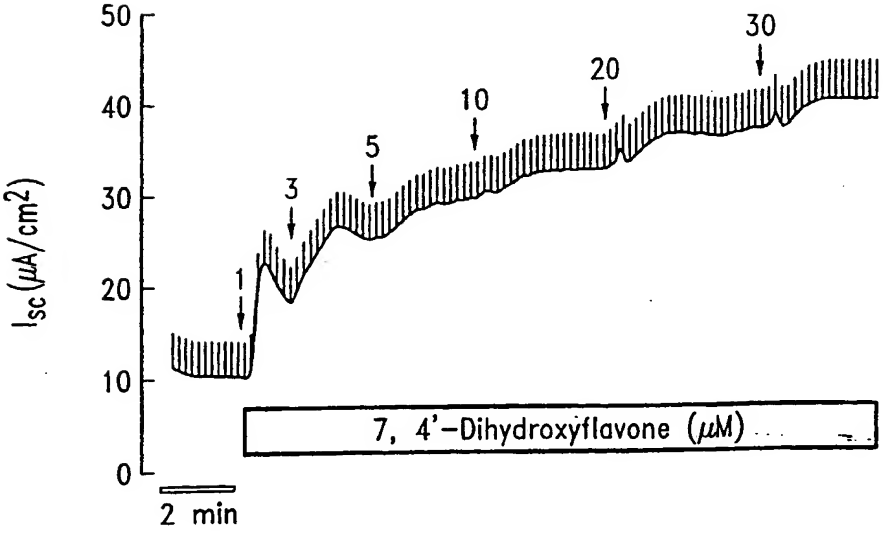


Fig. 13B

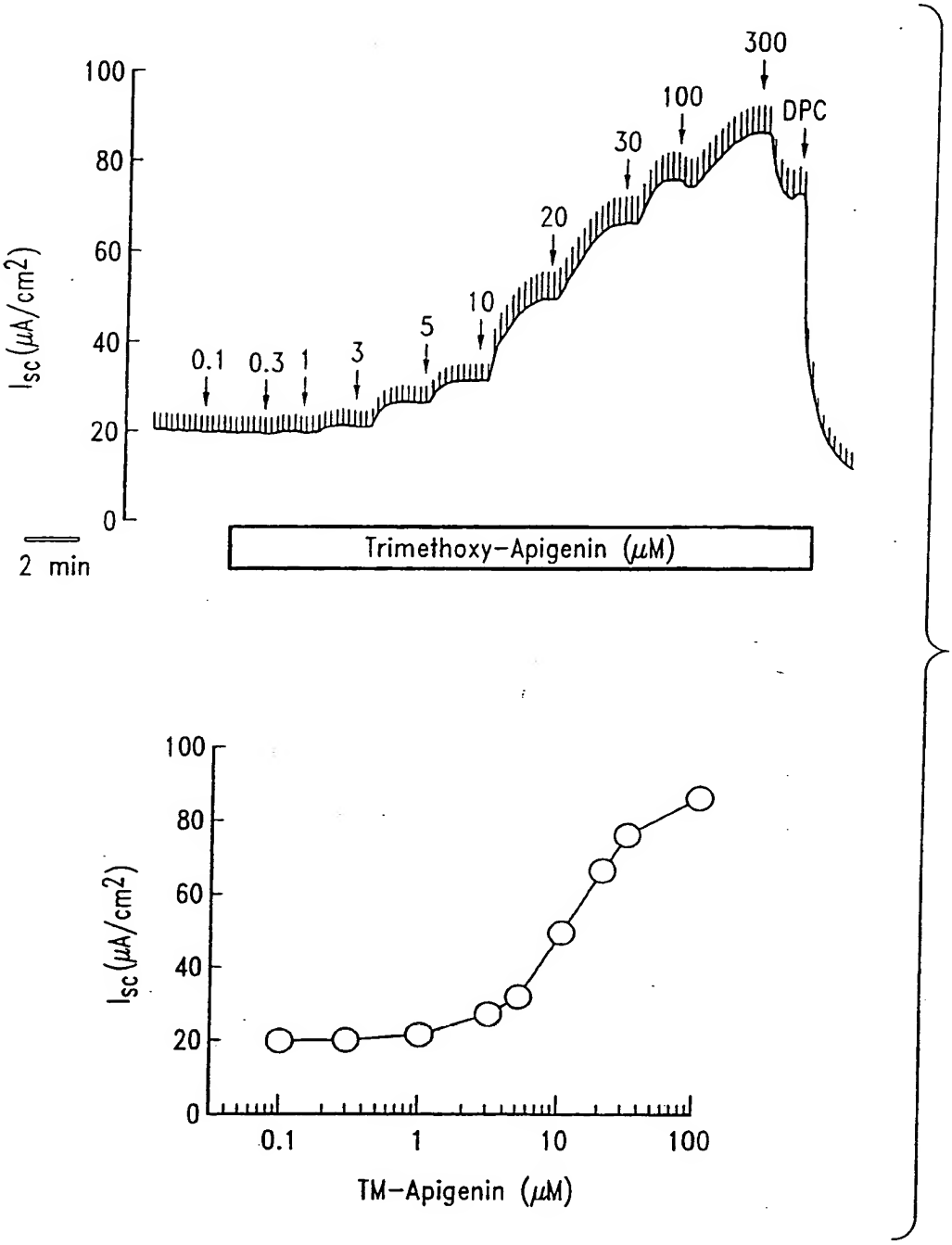


Fig. 13C

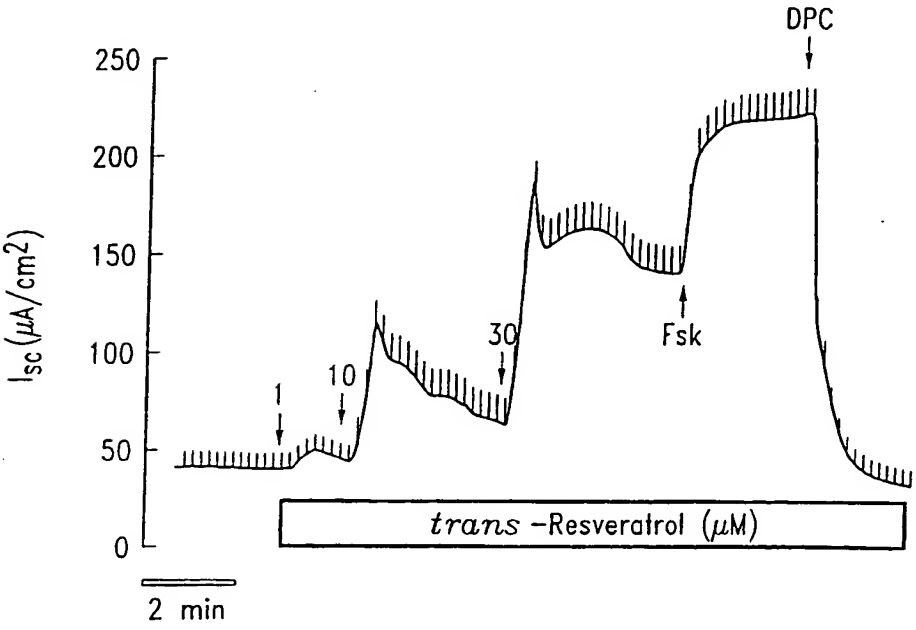

DPC
250
200
150
100
50
0
I_{sc} (μA/cm^2)
1
10
30
Fsk
trans -Resveratrol (μM)
2 min

Fig. 14

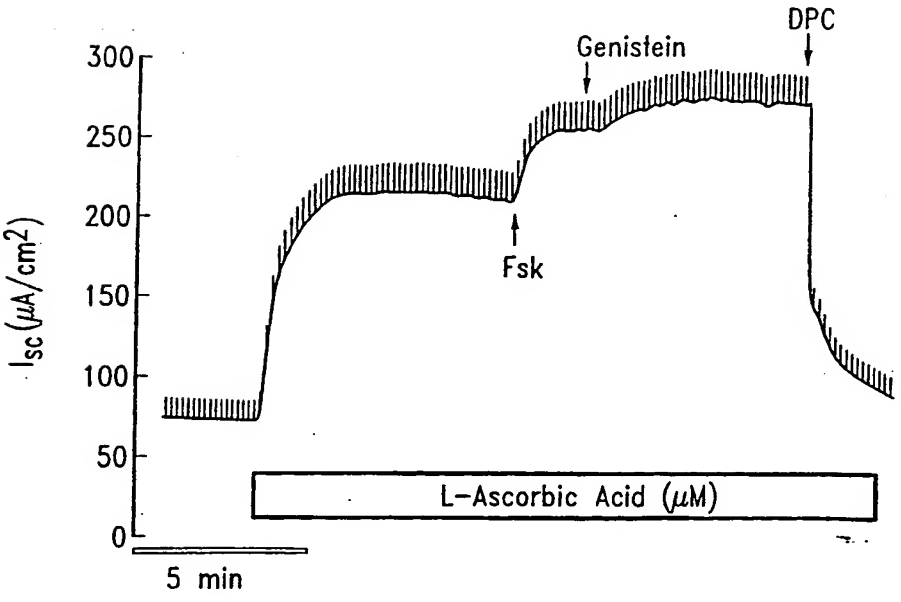

DPC
Genistein
300
250
200
150
100
50
0
I_{sc} (μA/cm^2)
Fsk
L-Ascorbic Acid (μM)
5 min

Fig. 15

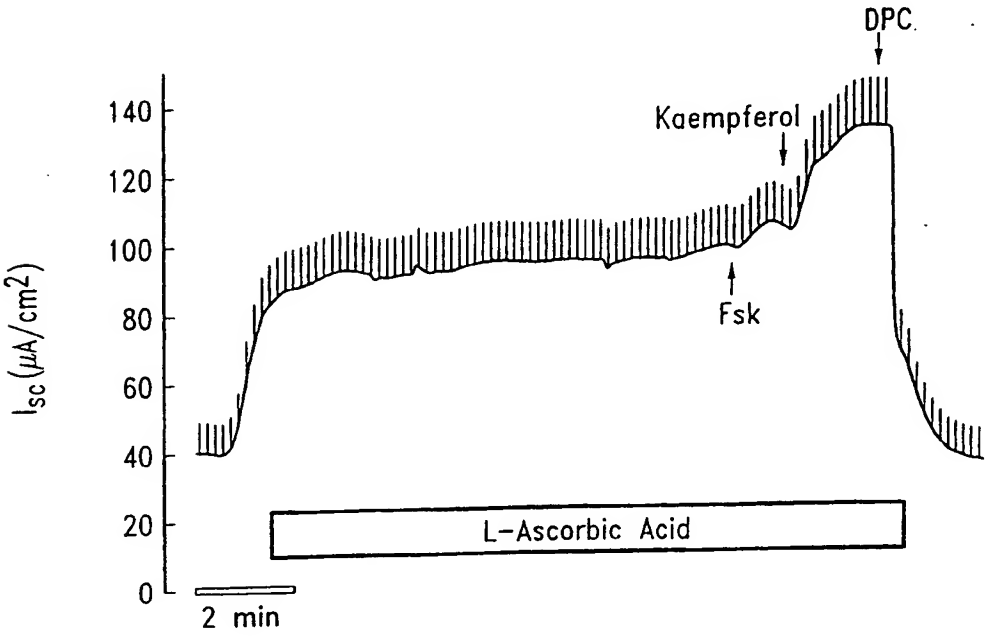


Fig. 16

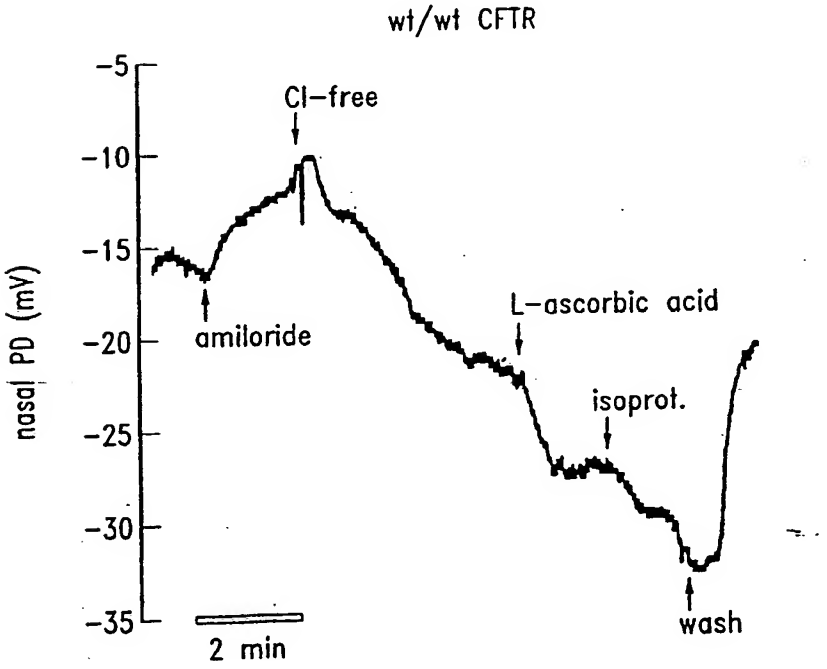


Fig. 17

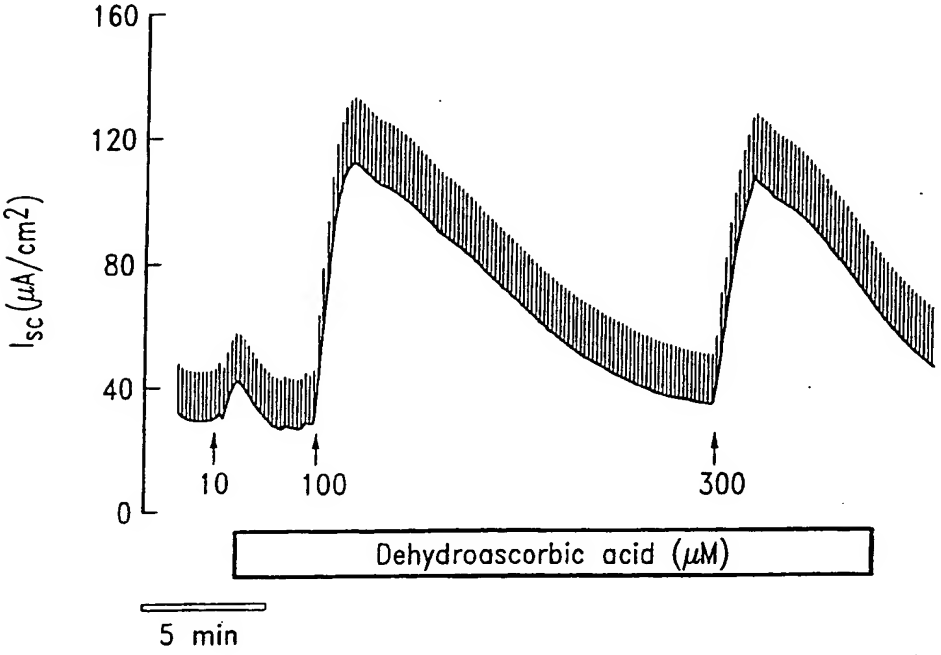


Fig. 18

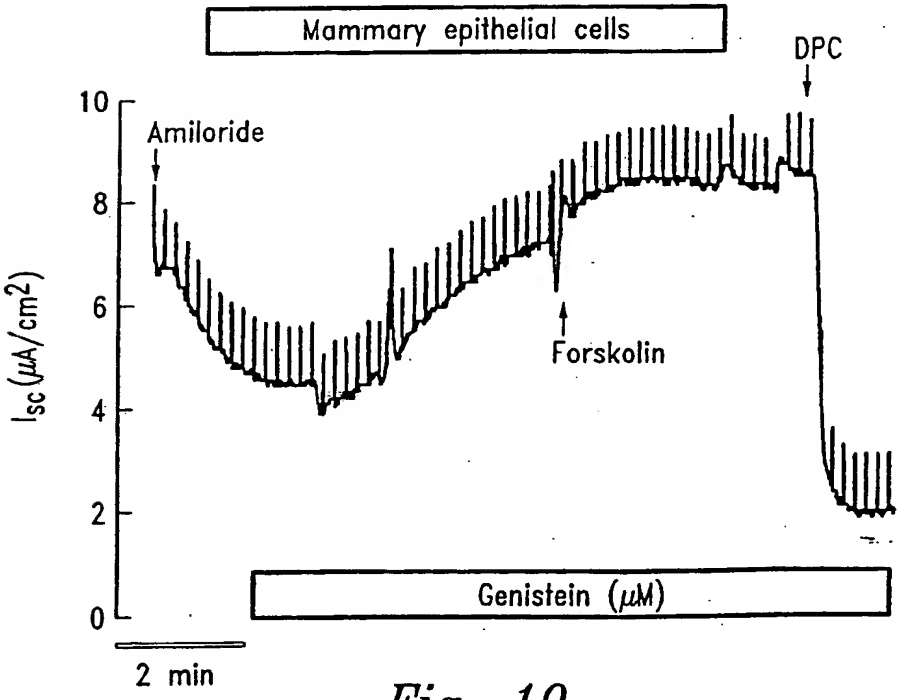


Fig. 19

SEQUENCE LISTING

<110> Fischer, Horst
Illek, Beate

<120> COMPOSITIONS AND METHODS FOR CYSTIC FIBROSIS THERAPY

<130> 200116.403PC

<140> US
<141> 1998-10-16

<150> US 08/951,912
<151> 1997-10-16

<160> 6

<170> PatentIn Ver. 2.0

<210> 1
<211> 6129
<212> DNA
<213> Homo sapiens

<400> 1
aattggaagc aaatgacatc acagcaggtc agagaaaaag ggttgagcgg caggcaccca 60
gagtagtagg tctttggcat taggagcttg agcccagacg gccctagcag ggaccccagc 120
gcccgagaga ccatgcagag gtcgcctctg gaaaaggcca gcgttgtctc caaacttttt 180
ttcagctgga ccagaccaat tttgaggaaa ggatacagac agcgcctgga attgtcagac 240
atataccaaa tcccttctgt tgattctgct gacaatctat ctgaaaaatt ggaaagagaa 300
tgggatagag agctggcttc aaagaaaaat cctaaactca ttaatgccct tcggcgatgt 360
ttttttctgga gatttatgtt ctatggaatc tttttatatt taggggaagt caccaaagca 420
gtacagcctc tcttactggg aagaatcata gcttcctatg acccggataa caaggaggaa 480
cgctctatcg cgatttatct aggcataggc ttatgccttc tctttattgt gaggacactg 540
ctcctacacc cagccatttt tggccttcat cacattggaa tgcagatgag aatagctatg 600
tttagtttga tttataagaa gactttaaag ctgtcaagcc gtgttctaga taaaataagt 660
attggacaac ttgttagtct cctttccaac aacctgaaca aatttgatga aggacttgca 720

ttggcacatt tcgtgtggat cgctcctttg caagtggcac tcctcatggg gctaatctgg 780
gagttgttac aggcgtctgc cttctgtgga cttggtttcc tgatagtcct tgccctttt 840
caggctgggc tagggagaat gatgatgaag tacagagatc agagagctgg gaagatcagt 900
gaaagacttg tgattacctc agaaatgatt gaaaatatcc aatctgttaa ggcatactgc 960
tgggaagaag caatggaaaa aatgattgaa aacttaagac aaacagaact gaaactgact 1020
cggaaggcag cctatgtgag atacttcaat agctcagcct tcttcttctc agggttcttt 1080
gtggtgtttt tatctgtgct tccctatgca ctaatcaaag gaatcatcct ccggaaaata 1140
ttcaccacca tctcattctg cattgttctg cgcatggcgg tcactcggca atttccctgg 1200
gctgtacaaa catggtatga ctctcttgga gcaataaaca aaatacagga tttcttacaa 1260
aagcaagaat ataagacatt ggaatataac ttaacgacta cagaagtagt gatggagaat 1320
gtaacagcct tctgggagga gggatttggg gaattatttg agaaagcaaa acaaaacaat 1380
aacaatagaa aaacttctaa tggtgatgac agcctcttct tcagtaattt ctcacttctt 1440
ggtactcctg tcctgaaaga tattaatttc aagatagaaa gaggacagtt gttggcggtt 1500
gctggatcca ctggagcagg caagacttca cttctaatga tgattatggg agaactggag 1560
ccttcagagg gtaaaattaa gcacagtgga agaatttcat tctgttctca gttttcctgg 1620
attatgcctg gcaccattaa agaaaatatc atctttggtg tttcctatga tgaatataga 1680
tacagaagcg tcatcaaagc atgccaacta gaagaggaca tctccaagtt tgcagagaaa 1740
gacaatatag ttcttggaga aggtggaatc acactgagtg gaggtcaacg agcaagaatt 1800
tctttagcaa gagcagtata caaagatgct gatttgtatt tattagactc tccttttgga 1860
tacctagatg ttttaacaga aaaagaaata tttgaaagct gtgtctgtaa actgatggct 1920
aacaaaacta ggattttggt cacttctaaa atggaacatt taaagaaagc tgacaaaata 1980
ttaattttga atgaaggtag cagctatttt tatgggacat tttcagaact ccaaaatcta 2040
cagccagact ttagctcaaa actcatggga tgtgattctt tcgaccaatt tagtgcagaa 2100
agaagaaatt caatcctaac tgagacctta caccgtttct cattagaagg agatgctcct 2160
gtctcctgga cagaaacaaa aaaacaatct tttaaacaga ctggagagtt tggggaaaaa 2220
aggaagaatt ctattctcaa tccaatcaac tctatacgaa aattttccat tgtgcaaaag 2280
actcccttac aaatgaatgg catcgaagag gattctgatg agcctttaga gagaaggctg 2340
tccttagtac cagattctga gcagggagag gcgatactgc ctcgcatcag cgtgatcagc 2400
actggcccca cgcttcaggc acgaaggagg cagtctgtcc tgaacctgat gacacactca 2460
gttaaccaag gtcagaacat tcaccgaaag acaacagcat ccacacgaaa agtgtcactg 2520
gcccctcagg caaacttgac tgaactggat atatattcaa gaaggttatc tcaagaaact 2580
ggcttggaaa taagtgaaga aattaacgaa gaagacttaa aggagtgcct ttttgatgat 2640
atggagagca taccagcagt gactacatgg aacacatacc ttcgatatat tactgtccac 2700
aagagcttaa tttttgtgct aatttggtgc ttagtaattt ttctggcaga ggtggctgct 2760
tctttggttg tgctgtggct ccttggaaac actcctcttc aagacaaagg gaatagtact 2820
catagtagaa ataacagcta tgcagtgatt atcaccagca ccagttcgta ttatgtgttt 2880
tacatttacg tgggagtagc cgacactttg cttgctatgg gattcttcag aggtctacca 2940

ctggtgcata ctctaatcac agtgtcgaaa attttacacc acaaaatgtt acattctgtt 3000
cttcaagcac ctatgtcaac cctcaacacg ttgaaagcag gtgggattct taatagattc 3060
tccaaagata tagcaatttt ggatgacctt ctgcctctta ccatatttga cttcatccag 3120
ttgttattaa ttgtgattgg agctatagca gttgtcgcag ttttacaacc ctacatcttt 3180
gttgcaacag tgccagtgat agtggctttt attatgttga gagcatattt cctccaaacc 3240
tcacagcaac tcaaacaact ggaatctgaa ggcaggagtc caattttcac tcatcttgtt 3300
acaagcttaa aaggactatg gacacttcgt gccttcggac ggcagcctta ctttgaaact 3360
ctgttccaca aagctctgaa tttacatact gccaactggt tcttgtacct gtcaacactg 3420
cgctggttcc aaatgagaat agaaatgatt tttgtcatct tcttcattgc tgttaccttc 3480
atttccattt taacaacagg agaaggagaa ggaagagttg gtattatcct gactttagcc 3540
atgaatatca tgagtacatt gcagtgggct gtaaactcca gcatagatgt ggatagcttg 3600
atgcgatctg tgagccgagt ctttaagttc attgacatgc caacagaagg taaacctacc 3660
aagtcaacca aaccatacaa gaatggccaa ctctcgaaag ttatgattat tgagaattca 3720
cacgtgaaga aagatgacat ctggccctca gggggccaaa tgactgtcaa agatctcaca 3780
gcaaaataca cagaaggtgg aaatgccata ttagagaaca tttccttctc aataagtcct 3840
ggccagaggg tgggcctctt gggaagaact ggatcaggga agagtacttt gttatcagct 3900
tttttgagac tactgaacac tgaaggagaa atccagatcg atggtgtgtc ttgggattca 3960
ataactttgc aacagtggag gaaagccttt ggagtgatac cacagaaagt atttattttt 4020
tctggaacat ttagaaaaaa cttggatccc tatgaacagt ggagtgatca agaaatatgg 4080
aaagttgcag atgaggttgg gctcagatct gtgatagaac agtttcctgg gaagcttgac 4140
tttgtccttg tggatggggg ctgtgtccta agccatggcc acaagcagtt gatgtgcttg 4200
gctagatctg ttctcagtaa ggcgaagatc ttgctgcttg atgaacccag tgctcatttg 4260
gatccagtaa cataccaaat aattagaaga actctaaaac aagcatttgc tgattgcaca 4320
gtaattctct gtgaacacag gatagaagca atgctggaat gccaacaatt tttggtcata 4380
gaagagaaca aagtgcggca gtacgattcc atccagaaac tgctgaacga gaggagcctc 4440
ttccggcaag ccatcagccc ctccgacagg gtgaagctct ttccccaccg gaactcaagc 4500
aagtgcaagt ctaagcccca gattgctgct ctgaaagagg agacagaaga agaggtgcaa 4560
gatacaaggc tttagagagc agcataaatg ttgacatggg acatttgctc atggaattgg 4620
agctcgtggg acagtcacct catggaattg gagctcgtgg aacagttacc tctgcctcag 4680
aaaacaagga tgaattaagt tttttttaa aaaagaaaca tttggtaagg ggaattgagg 4740
acactgatat gggtcttgat aaatggcttc ctggcaatag tcaaattgtg tgaaaggtac 4800
ttcaaatcct tgaagattta ccacttgtgt tttgcaagcc agattttcct gaaaaccctt 4860
gccatgtgct agtaattgga aaggcagctc taaatgtcaa tcagcctagt tgatcagctt 4920
attgtctagt gaaactcgtt aatttgtagt gttggagaag aactgaaatc atacttctta 4980
gggttatgat taagtaatga taactggaaa cttcagcggt ttatataagc ttgtattcct 5040
ttttctctcc tctccccatg atgtttagaa acacaactat attgtttgct aagcattcca 5100
actatctcat ttccaagcaa gtattagaat accacaggaa ccacaagact gcacatcaaa 5160

atatgcccca ttcaacatct agtgagcagt caggaaagag aacttccaga tcctggaaat 5220
cagggttagt attgtccagg tctaccaaaa atctcaatat ttcagataat cacaatacat 5280
cccttacctg ggaaagggct gttataatct ttcacagggg acaggatggt tcccttgatg 5340
aagaagttga tatgcctttt cccaactcca gaaagtgaca agctcacaga cctttgaact 5400
agagtttagc tggaaaagta tgttagtgca aattgtcaca ggacagccct tctttccaca 5460
gaagctccag gtagagggtg tgtaagtaga taggccatgg gcactgtggg tagacacaca 5520
tgaagtccaa gcatttagat gtataggttg atggtggtat gttttcaggc tagatgtatg 5580
tacttcatgc tgtctacact aagagagaat gagagacaca ctgaagaagc accaatcatg 5640
aattagtttt atatgcttct gttttataat tttgtgaagc aaaatttttt ctctaggaaa 5700
tatttatttt aataatgttt caaacatata ttacaatgct gtattttaaa agaatgatta 5760
tgaattacat ttgtataaaa taatttttat atttgaaata ttgacttttt atggcactag 5820
tatttttatg aaatattatg ttaaaactgg gacaggggag aacctagggt gatattaacc 5880
aggggccatg aatcaccttt tggtctggag ggaagccttg gggctgatcg agttgttgcc 5940
cacagctgta tgattcccag ccagacacag cctcttagat gcagttctga agaagatggt 6000
accaccagtc tgactgtttc catcaagggt acactgcctt ctcaactcca aactgactct 6060
taagaagact gcattatatt tattactgta agaaaatatc acttgtcaat aaaatccata 6120
catttgtgt 6129

<210> 2
<211> 1480
<212> PRT
<213> Homo sapiens

<400> 2
Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val Ser Lys Leu Phe
1 5 10 15

Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr Arg Gln Arg Leu
20 25 30

Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser Ala Asp Asn
35 40 45

Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu Ala Ser Lys
50 55 60

Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe Phe Trp Arg

65 70 75 80

Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val Thr Lys Ala
85 90 95

Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr Asp Pro Asp
100 105 110

Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile Gly Leu Cys
115 120 125

Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro Ala Ile Phe Gly
130 135 140

Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe Ser Leu Ile
145 150 155 160

Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp Lys Ile Ser
165 170 175

Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn Lys Phe Asp
180 185 190

Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro Leu Gln Val
195 200 205

Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala Ser Ala Phe
210 215 220

Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln Ala Gly Leu
225 230 235 240

Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly Lys Ile Ser
245 250 255

Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile Gln Ser Val
260 265 270

Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile Glu Asn Leu
275 280 285

Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr Val Arg Tyr
290 295 300

Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val Val Phe Leu
305 310 315 320

Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu Arg Lys Ile
325 330 335

Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala Val Thr Arg
340 345 350

Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu Gly Ala Ile
355 360 365

Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys Thr Leu Glu
370 375 380

Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val Thr Ala Phe
385 390 395 400

Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys Gln Asn Asn
405 410 415

Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu Phe Phe Ser Asn
420 425 430

Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn Phe Lys Ile
435 440 445

Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr Gly Ala Gly Lys
450 455 460

Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu Pro Ser Glu Gly
465 470 475 480

Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln Phe Ser Trp
485 490 495

Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr
500 505 510

Asp Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu
515 520 525

Asp Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly
530 535 540

Gly Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg
545 550 555 560

Ala Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser Pro Phe Gly
565 570 575

Tyr Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser Cys Val Cys
580 585 590

Lys Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser Lys Met Glu
595 600 605

His Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu Gly Ser Ser
610 615 620

Tyr Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln Pro Asp Phe
625 630 635 640

Ser Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe Ser Ala Glu
645 650 655

Arg Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe Ser Leu Glu

660 665 670

Gly Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln Ser Phe Lys
675 680 685

Gln Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile Leu Asn Pro
690 695 700

Ile Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr Pro Leu Gln
705 710 715 720

Met Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu Arg Arg Leu
725 730 735

Ser Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile Leu Pro Arg Ile
740 745 750

Ser Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg Arg Gln Ser
755 760 765

Val Leu Asn Leu Met Thr His Ser Val Asn Gln Gly Gln Asn Ile His
770 775 780

Arg Lys Thr Thr Ala Ser Thr Arg Lys Val Ser Leu Ala Pro Gln Ala
785 790 795 800

Asn Leu Thr Glu Leu Asp Ile Tyr Ser Arg Arg Leu Ser Gln Glu Thr
805 810 815

Gly Leu Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp Leu Lys Glu Cys
820 825 830

Leu Phe Asp Asp Met Glu Ser Ile Pro Ala Val Thr Thr Trp Asn Thr
835 840 845

Tyr Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile Phe Val Leu Ile
850 855 860

Trp Cys Leu Val Ile Phe Leu Ala Glu Val Ala Ala Ser Leu Val Val
865 870 875 880

Leu Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys Gly Asn Ser Thr
885 890 895

His Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr Ser Thr Ser Ser
900 905 910

Tyr Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp Thr Leu Leu Ala
915 920 925

Met Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr Leu Ile Thr Val
930 935 940

Ser Lys Ile Leu His His Lys Met Leu His Ser Val Leu Gln Ala Pro
945 950 955 960

Met Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile Leu Asn Arg Phe
965 970 975

Ser Lys Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro Leu Thr Ile Phe
980 985 990

Asp Phe Ile Gln Leu Leu Leu Ile Val Ile Gly Ala Ile Ala Val Val
995 1000 1005

Ala Val Leu Gln Pro Tyr Ile Phe Val Ala Thr Val Pro Val Ile Val
1010 1015 1020

Ala Phe Ile Met Leu Arg Ala Tyr Phe Leu Gln Thr Ser Gln Gln Leu
1025 1030 1035 1040

Lys Gln Leu Glu Ser Glu Gly Arg Ser Pro Ile Phe Thr His Leu Val
1045 1050 1055

Thr Ser Leu Lys Gly Leu Trp Thr Leu Arg Ala Phe Gly Arg Gln Pro
1060 1065 1070

Tyr Phe Glu Thr Leu Phe His Lys Ala Leu Asn Leu His Thr Ala Asn
1075 1080 1085

Trp Phe Leu Tyr Leu Ser Thr Leu Arg Trp Phe Gln Met Arg Ile Glu
1090 1095 1100

Met Ile Phe Val Ile Phe Phe Ile Ala Val Thr Phe Ile Ser Ile Leu
1105 1110 1115 1120

Thr Thr Gly Glu Gly Glu Gly Arg Val Gly Ile Ile Leu Thr Leu Ala
1125 1130 1135

Met Asn Ile Met Ser Thr Leu Gln Trp Ala Val Asn Ser Ser Ile Asp
1140 1145 1150

Val Asp Ser Leu Met Arg Ser Val Ser Arg Val Phe Lys Phe Ile Asp
1155 1160 1165

Met Pro Thr Glu Gly Lys Pro Thr Lys Ser Thr Lys Pro Tyr Lys Asn
1170 1175 1180

Gly Gln Leu Ser Lys Val Met Ile Ile Glu Asn Ser His Val Lys Lys
1185 1190 1195 1200

Asp Asp Ile Trp Pro Ser Gly Gly Gln Met Thr Val Lys Asp Leu Thr
1205 1210 1215

Ala Lys Tyr Thr Glu Gly Gly Asn Ala Ile Leu Glu Asn Ile Ser Phe
1220 1225 1230

Ser Ile Ser Pro Gly Gln Arg Val Gly Leu Leu Gly Arg Thr Gly Ser
1235 1240 1245

Gly Lys Ser Thr Leu Leu Ser Ala Phe Leu Arg Leu Leu Asn Thr Glu

1250 1255 1260

Gly Glu Ile Gln Ile Asp Gly Val Ser Trp Asp Ser Ile Thr Leu Gln
1265 1270 1275 1280

Gln Trp Arg Lys Ala Phe Gly Val Ile Pro Gln Lys Val Phe Ile Phe
1285 1290 1295

Ser Gly Thr Phe Arg Lys Asn Leu Asp Pro Tyr Glu Gln Trp Ser Asp
1300 1305 1310

Gln Glu Ile Trp Lys Val Ala Asp Glu Val Gly Leu Arg Ser Val Ile
1315 1320 1325

Glu Gln Phe Pro Gly Lys Leu Asp Phe Val Leu Val Asp Gly Gly Cys
1330 1335 1340

Val Leu Ser His Gly His Lys Gln Leu Met Cys Leu Ala Arg Ser Val
1345 1350 1355 1360

Leu Ser Lys Ala Lys Ile Leu Leu Leu Asp Glu Pro Ser Ala His Leu
1365 1370 1375

Asp Pro Val Thr Tyr Gln Ile Ile Arg Arg Thr Leu Lys Gln Ala Phe
1380 1385 1390

Ala Asp Cys Thr Val Ile Leu Cys Glu His Arg Ile Glu Ala Met Leu
1395 1400 1405

Glu Cys Gln Gln Phe Leu Val Ile Glu Glu Asn Lys Val Arg Gln Tyr
1410 1415 1420

Asp Ser Ile Gln Lys Leu Leu Asn Glu Arg Ser Leu Phe Arg Gln Ala
1425 1430 1435 1440

Ile Ser Pro Ser Asp Arg Val Lys Leu Phe Pro His Arg Asn Ser Ser
1445 1450 1455

Lys Cys Lys Ser Lys Pro Gln Ile Ala Ala Leu Lys Glu Glu Thr Glu
1460 1465 1470

Glu Glu Val Gln Asp Thr Arg Leu
1475 1480

<210> 3
<211> 6126
<212> DNA
<213> Homo sapiens

<400> 3
aattggaagc aaatgacatc acagcaggtc agagaaaaag ggttgagcgg caggcaccca 60
gagtagtagg tctttggcat taggagcttg agcccagacg gccctagcag ggaccccagc 120
gcccgagaga ccatgcagag gtcgcctctg gaaaaggcca gcgttgtctc caaacttttt 180
ttcagctgga ccagaccaat tttgaggaaa ggatacagac agcgcctgga attgtcagac 240
atataccaaa tcccttctgt tgattctgct gacaatctat ctgaaaaatt ggaaagagaa 300
tgggatagag agctggcttc aaagaaaaat cctaaactca ttaatgccct tcggcgatgt 360
tttttctgga gatttatgtt ctatggaatc tttttatatt taggggaagt caccaaagca 420
gtacagcctc tcttactggg aagaatcata gcttcctatg acccggataa caaggaggaa 480
cgctctatcg cgatttatct aggcataggc ttatgccttc tctttattgt gaggacactg 540
ctcctacacc cagccatttt tggccttcat cacattggaa tgcagatgag aatagctatg 600
tttagtttga tttataagaa gactttaaag ctgtcaagcc gtgttctaga taaaataagt 660
attggacaac ttgttagtct cctttccaac aacctgaaca aatttgatga aggacttgca 720
ttggcacatt tcgtgtggat cgctcctttg caagtggcac tcctcatggg gctaatctgg 780
gagttgttac aggcgtctgc cttctgtgga cttggtttcc tgatagtcct tgccctttt 840
caggctgggc tagggagaat gatgatgaag tacagagatc agagagctgg gaagatcagt 900
gaaagacttg tgattacctc agaaatgatt gaaaatatcc aatctgttaa ggcatactgc 960
tgggaagaag caatggaaaa aatgattgaa aacttaagac aaacagaact gaaactgact 1020
cggaaggcag cctatgtgag atacttcaat agctcagcct tcttcttctc agggttcttt 1080
gtggtgtttt tatctgtgct tccctatgca ctaatcaaag gaatcatcct ccggaaaata 1140
ttcaccacca tctcattctg cattgttctg cgcatggcgg tcactcggca atttccctgg 1200
gctgtacaaa catggtatga ctctcttgga gcaataaaca aaatacagga tttcttacaa 1260
aagcaagaat ataagacatt ggaatataac ttaacgacta cagaagtagt gatggagaat 1320
gtaacagcct tctgggagga gggatttggg gaattatttg agaaagcaaa acaaaacaat 1380

aacaatagaa aaacttctaa tggtgatgac agcctcttct tcagtaattt ctcacttctt 1440
ggtactcctg tcctgaaaga tattaatttc aagatagaaa gaggacagtt gttggcggtt 1500
gctggatcca ctggagcagg caagacttca cttctaatga tgattatggg agaactggag 1560
ccttcagagg gtaaaattaa gcacagtgga agaatttcat tctgttctca gttttcctgg 1620
attatgcctg gcaccattaa agaaaatatc atcggtgttt cctatgatga atatagatac 1680
agaagcgtca tcaaagcatg ccaactagaa gaggacatct ccaagtttgc agagaaagac 1740
aatatagttc ttggagaagg tggaatcaca ctgagtggag gtcaacgagc aagaatttct 1800
ttagcaagag cagtatacaa agatgctgat ttgtatttat tagactctcc ttttggatac 1860
ctagatgttt taacagaaaa agaaatattt gaaagctgtg tctgtaaact gatggctaac 1920
aaaactagga ttttggtcac ttctaaaatg gaacatttaa agaaagctga caaaatatta 1980
attttgaatg aaggtagcag ctatttttat gggacatttt cagaactcca aaatctacag 2040
ccagacttta gctcaaaact catgggatgt gattctttcg accaatttag tgcagaaaga 2100
agaaattcaa tcctaactga gaccttacac cgtttctcat tagaaggaga tgctcctgtc 2160
tcctggacag aaacaaaaaa acaatctttt aaacagactg gagagtttgg ggaaaaaagg 2220
aagaattcta ttctcaatcc aatcaactct atacgaaaat tttccattgt gcaaaagact 2280
cccttacaaa tgaatggcat cgaagaggat tctgatgagc ctttagagag aaggctgtcc 2340
ttagtaccag attctgagca gggagaggcg atactgcctc gcatcagcgt gatcagcact 2400
ggccccacgc ttcaggcacg aaggaggcag tctgtcctga acctgatgac acactcagtt 2460
aaccaaggtc agaacattca ccgaaagaca acagcatcca cacgaaaagt gtcactggcc 2520
cctcaggcaa acttgactga actggatata tattcaagaa ggttatctca agaaactggc 2580
ttggaaataa gtgaagaaat taacgaagaa gacttaaagg agtgcctttt tgatgatatg 2640
gagagcatac cagcagtgac tacatggaac acataccttc gatatattac tgtccacaag 2700
agcttaattt ttgtgctaat ttggtgctta gtaatttttc tggcagaggt ggctgcttct 2760
ttggttgtgc tgtggctcct tggaaacact cctcttcaag acaaagggaa tagtactcat 2820
agtagaaata acagctatgc agtgattatc accagcacca gttcgtatta tgtgttttac 2880
atttacgtgg gagtagccga cactttgctt gctatgggat tcttcagagg tctaccactg 2940
gtgcatactc taatcacagt gtcgaaaatt ttacaccaca aaatgttaca ttctgttctt 3000
caagcaccta tgtcaaccct caacacgttg aaagcaggtg ggattcttaa tagattctcc 3060
aaagatatag caatttttga tgaccttctg cctcttacca tatttgactt catccagttg 3120
ttattaattg tgattggagc tatagcagtt gtcgcagttt tacaacccta catctttgtt 3180
gcaacagtgc cagtgatagt ggcttttatt atgttgagag catatttcct ccaaacctca 3240
cagcaactca aacaactgga atctgaaggc aggagtccaa ttttcactca tcttgttaca 3300
agcttaaaag gactatggac acttcgtgcc ttcggacggc agccttactt tgaaactctg 3360
ttccacaaag ctctgaattt acatactgcc aactggttct tgtacctgtc aacactgcgc 3420
tggttccaaa tgagaataga aatgattttt gtcatcttct tcattgctgt taccttcatt 3480
tccatttttaa caacaggaga aggagaagga agagttggta ttatcctgac tttagccatg 3540
aatatcatga gtacattgca gtgggctgta aactccagca tagatgtgga tagcttgatg 3600

cgatctgtga gccgagtctt taagttcatt gacatgccaa cagaaggtaa acctaccaag 3660
tcaaccaaac catacaagaa tggccaactc tcgaaagtta tgattattga gaattcacac 3720
gtgaagaaag atgacatctg gccctcaggg ggccaaatga ctgtcaaaga tctcacagca 3780
aaatacacag aaggtggaaa tgccatatta gagaacattt ccttctcaat aagtcctggc 3840
cagagggtgg gcctcttggg aagaactgga tcagggaaga gtactttgtt atcagctttt 3900
ttgagactac tgaacactga aggagaaatc cagatcgatg gtgtgtcttg ggattcaata 3960
actttgcaac agtggaggaa agcctttgga gtgataccac agaaagtatt tatttttct 4020
ggaacattta gaaaaaactt ggatccctat gaacagtgga gtgatcaaga aatatggaaa 4080
gttgcagatg aggttgggct cagatctgtg atagaacagt ttcctgggaa gcttgacttt 4140
gtccttgtgg atgggggctg tgtcctaagc catggccaca agcagttgat gtgcttggct 4200
agatctgttc tcagtaaggc gaagatcttg ctgcttgatg aacccagtgc tcatttggat 4260
ccagtaacat accaaataat tagaagaact ctaaaacaag catttgctga ttgcacagta 4320
attctctgtg aacacaggat agaagcaatg ctggaatgcc aacaattttt ggtcatagaa 4380
gagaacaaag tgcggcagta cgattccatc cagaaactgc tgaacgagag gagcctcttc 4440
cggcaagcca tcagcccctc cgacagggtg aagctctttc cccaccggaa ctcaagcaag 4500
tgcaagtcta agccccagat tgctgctctg aaagaggaga cagaagaaga ggtgcaagat 4560
acaaggcttt agagagcagc ataaatgttg acatgggaca tttgctcatg gaattggagc 4620
tcgtgggaca gtcacctcat ggaattggag ctcgtggaac agttacctct gcctcagaaa 4680
acaaggatga attaagtttt tttttaaaaa agaaacattt ggtaagggga attgaggaca 4740
ctgatatggg tcttgataaa tggcttcctg gcaatagtca aattgtgtga aaggtacttc 4800
aaatccttga agatttacca cttgtgtttt gcaagccaga ttttcctgaa aacccttgcc 4860
atgtgctagt aattggaaag gcagctctaa atgtcaatca gcctagttga tcagcttatt 4920
gtctagtgaa actcgttaat ttgtagtgtt ggagaagaac tgaaatcata cttcttaggg 4980
ttatgattaa gtaatgataa ctggaaactt cagcggttta tataagcttg tattcctttt 5040
tctctcctct ccccatgatg tttagaaaca caactatatt gtttgctaag cattccaact 5100
atctccatttc caagcaagta ttagaatacc acaggaacca caagactgca catcaaaata 5160
tgccccattc aacatctagt gagcagtcag gaaagagaac ttccagatcc tggaaatcag 5220
ggttagtatt gtccaggtct accaaaaatc tcaatatttc agataatcac aatacatccc 5280
ttacctggga aagggctgtt ataatctttc acaggggaca ggatggttcc cttgatgaag 5340
aagttgatat gccttttccc aactccagaa agtgacaagc tcacagacct ttgaactaga 5400
gtttagctgg aaaagtatgt tagtgcaaat tgtcacagga cagcccttct ttccacagaa 5460
gctccaggta gagggtgtgt aagtagatag gccatgggca ctgtgggtag acacacatga 5520
agtccaagca tttagatgta taggttgatg gtggtatgtt ttcaggctag atgtatgtac 5580
ttcatgctgt ctacactaag agagaatgag agacacactg aagaagcacc aatcatgaat 5640
tagttttata tgcttctgtt ttataatttt gtgaagcaaa attttttctc taggaaatat 5700
ttattttaat aatgtttcaa acatatatta caatgctgta ttttaaaaga atgattatga 5760
attacatttg tataaaataa tttttatatt tgaaatattg acttttttatg gcactagtat 5820

ttttatgaaa tattatgtta aaactgggac aggggagaac ctagggtgat attaaccagg 5880
ggccatgaat caccttttgg tctggaggga agccttgggg ctgatcgagt tgttgcccac 5940
agctgtatga ttcccagcca gacacagcct cttagatgca gttctgaaga agatggtacc 6000
accagtctga ctgtttccat caagggtaca ctgccttctc aactccaaac tgactcttaa 6060
gaagactgca ttatatttat tactgtaaga aaatatcact tgtcaataaa atccatacat 6120
ttgtgt 6126

<210> 4
<211> 1479
<212> PRT
<213> Homo sapiens

<400> 4

Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val Ser Lys Leu Phe
1 5 10 15

Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr Arg Gln Arg Leu
20 25 30

Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser Ala Asp Asn
35 40 45

Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu Ala Ser Lys
50 55 60

Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe Phe Trp Arg
65 70 75 80

Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val Thr Lys Ala
85 90 95

Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr Asp Pro Asp
100 105 110

Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile Gly Leu Cys
115 120 125

Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro Ala Ile Phe Gly
130 135 140

Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe Ser Leu Ile
145 150 155 160

Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp Lys Ile Ser
165 170 175

Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn Lys Phe Asp
180 185 190

Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro Leu Gln Val
195 200 205

Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala Ser Ala Phe
210 215 220

Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln Ala Gly Leu
225 230 235 240

Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly Lys Ile Ser
245 250 255

Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile Gln Ser Val
260 265 270

Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile Glu Asn Leu
275 280 285

Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr Val Arg Tyr
290 295 300

Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val Val Phe Leu
305 310 315 320

Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu Arg Lys Ile

325 330 335

Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala Val Thr Arg
340 345 350

Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu Gly Ala Ile
355 360 365

Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys Thr Leu Glu
370 375 380

Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val Thr Ala Phe
385 390 395 400

Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys Gln Asn Asn
405 410 415

Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu Phe Phe Ser Asn
420 425 430

Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn Phe Lys Ile
435 440 445

Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr Gly Ala Gly Lys
450 455 460

Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu Pro Ser Glu Gly
465 470 475 480

Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln Phe Ser Trp
485 490 495

Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Gly Val Ser Tyr Asp
500 505 510

Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp
515 520 525

Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly Gly
530 535 540

Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg Ala
545 550 555 560

Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser Pro Phe Gly Tyr
565 570 575

Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser Cys Val Cys Lys
580 585 590

Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser Lys Met Glu His
595 600 605

Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu Gly Ser Ser Tyr
610 615 620

Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln Pro Asp Phe Ser
625 630 635 640

Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe Ser Ala Glu Arg
645 650 655

Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe Ser Leu Glu Gly
660 665 670

Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln Ser Phe Lys Gln
675 680 685

Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile Leu Asn Pro Ile
690 695 700

Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr Pro Leu Gln Met
705 710 715 720

Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu Arg Arg Leu Ser
725 730 735

Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile Leu Pro Arg Ile Ser
740 745 750

Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg Arg Gln Ser Val
755 760 765

Leu Asn Leu Met Thr His Ser Val Asn Gln Gly Gln Asn Ile His Arg
770 775 780

Lys Thr Thr Ala Ser Thr Arg Lys Val Ser Leu Ala Pro Gln Ala Asn
785 790 795 800

Leu Thr Glu Leu Asp Ile Tyr Ser Arg Arg Leu Ser Gln Glu Thr Gly
805 810 815

Leu Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp Leu Lys Glu Cys Leu
820 825 830

Phe Asp Asp Met Glu Ser Ile Pro Ala Val Thr Thr Trp Asn Thr Tyr
835 840 845

Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile Phe Val Leu Ile Trp
850 855 860

Cys Leu Val Ile Phe Leu Ala Glu Val Ala Ala Ser Leu Val Val Leu
865 870 875 880

Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys Gly Asn Ser Thr His
885 890 895

Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr Ser Thr Ser Ser Tyr
900 905 910

Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp Thr Leu Leu Ala Met

915 920 925

Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr Leu Ile Thr Val Ser
930 935 940

Lys Ile Leu His His Lys Met Leu His Ser Val Leu Gln Ala Pro Met
945 950 955 960

Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile Leu Asn Arg Phe Ser
965 970 975

Lys Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro Leu Thr Ile Phe Asp
980 985 990

Phe Ile Gln Leu Leu Leu Ile Val Ile Gly Ala Ile Ala Val Val Ala
995 1000 1005

Val Leu Gln Pro Tyr Ile Phe Val Ala Thr Val Pro Val Ile Val Ala
1010 1015 1020

Phe Ile Met Leu Arg Ala Tyr Phe Leu Gln Thr Ser Gln Gln Leu Lys
1025 1030 1035 1040

Gln Leu Glu Ser Glu Gly Arg Ser Pro Ile Phe Thr His Leu Val Thr
1045 1050 1055

Ser Leu Lys Gly Leu Trp Thr Leu Arg Ala Phe Gly Arg Gln Pro Tyr
1060 1065 1070

Phe Glu Thr Leu Phe His Lys Ala Leu Asn Leu His Thr Ala Asn Trp
1075 1080 1085

Phe Leu Tyr Leu Ser Thr Leu Arg Trp Phe Gln Met Arg Ile Glu Met
1090 1095 1100

Ile Phe Val Ile Phe Phe Ile Ala Val Thr Phe Ile Ser Ile Leu Thr
1105 1110 1115 1120

Thr Gly Glu Gly Glu Gly Arg Val Gly Ile Ile Leu Thr Leu Ala Met
1125 1130 1135

Asn Ile Met Ser Thr Leu Gln Trp Ala Val Asn Ser Ser Ile Asp Val
1140 1145 1150

Asp Ser Leu Met Arg Ser Val Ser Arg Val Phe Lys Phe Ile Asp Met
1155 1160 1165

Pro Thr Glu Gly Lys Pro Thr Lys Ser Thr Lys Pro Tyr Lys Asn Gly
1170 1175 1180

Gln Leu Ser Lys Val Met Ile Ile Glu Asn Ser His Val Lys Lys Asp
1185 1190 1195 1200

Asp Ile Trp Pro Ser Gly Gly Gln Met Thr Val Lys Asp Leu Thr Ala
1205 1210 1215

Lys Tyr Thr Glu Gly Gly Asn Ala Ile Leu Glu Asn Ile Ser Phe Ser
1220 1225 1230

Ile Ser Pro Gly Gln Arg Val Gly Leu Leu Gly Arg Thr Gly Ser Gly
1235 1240 1245

Lys Ser Thr Leu Leu Ser Ala Phe Leu Arg Leu Leu Asn Thr Glu Gly
1250 1255 1260

Glu Ile Gln Ile Asp Gly Val Ser Trp Asp Ser Ile Thr Leu Gln Gln
1265 1270 1275 1280

Trp Arg Lys Ala Phe Gly Val Ile Pro Gln Lys Val Phe Ile Phe Ser
1285 1290 1295

Gly Thr Phe Arg Lys Asn Leu Asp Pro Tyr Glu Gln Trp Ser Asp Gln
1300 1305 1310

Glu Ile Trp Lys Val Ala Asp Glu Val Gly Leu Arg Ser Val Ile Glu
1315 1320 1325

Gln Phe Pro Gly Lys Leu Asp Phe Val Leu Val Asp Gly Gly Cys Val
1330 1335 1340

Leu Ser His Gly His Lys Gln Leu Met Cys Leu Ala Arg Ser Val Leu
1345 1350 1355 1360

Ser Lys Ala Lys Ile Leu Leu Leu Asp Glu Pro Ser Ala His Leu Asp
1365 1370 1375

Pro Val Thr Tyr Gln Ile Ile Arg Arg Thr Leu Lys Gln Ala Phe Ala
1380 1385 1390

Asp Cys Thr Val Ile Leu Cys Glu His Arg Ile Glu Ala Met Leu Glu
1395 1400 1405

Cys Gln Gln Phe Leu Val Ile Glu Glu Asn Lys Val Arg Gln Tyr Asp
1410 1415 1420

Ser Ile Gln Lys Leu Leu Asn Glu Arg Ser Leu Phe Arg Gln Ala Ile
1425 1430 1435 1440

Ser Pro Ser Asp Arg Val Lys Leu Phe Pro His Arg Asn Ser Ser Lys
1445 1450 1455

Cys Lys Ser Lys Pro Gln Ile Ala Ala Leu Lys Glu Glu Thr Glu Glu
1460 1465 1470

Glu Val Gln Asp Thr Arg Leu
1475

<210> 5
<211> 6129
<212> DNA

<213> Homo sapiens

<400> 5

aattggaagc aaatgacatc acagcaggtc agagaaaaag ggttgagcgg caggcaccca 60
gagtagtagg tctttggcat taggagcttg agcccagacg gccctagcag ggaccccagc 120
gcccgagaga ccatgcagag gtcgcctctg gaaaaggcca gcgttgtctc caaacttttt 180
ttcagctgga ccagaccaat tttgaggaaa ggatacagac agcgcctgga attgtcagac 240
atataccaaa tcccttctgt tgattctgct gacaatctat ctgaaaaatt ggaaagagaa 300
tgggatagag agctggcttc aaagaaaaat cctaaactca ttaatgccct tcggcgatgt 360
tttttctgga gatttatgtt ctatggaatc tttttatatt taggggaagt caccaaagca 420
gtacagcctc tcttactggg aagaatcata gcttcctatg acccggataa caaggaggaa 480
cgctctatcg cgatttatct aggcataggc ttatgccttc tctttattgt gaggacactg 540
ctcctacacc cagccatttt tggccttcat cacattggaa tgcagatgag aatagctatg 600
tttagtttga tttataagaa gactttaaag ctgtcaagcc gtgttctaga taaaataagt 660
attggacaac ttgttagtct cctttccaac aacctgaaca aatttgatga aggacttgca 720
ttggcacatt tcgtgtggat cgctcctttg caagtggcac tcctcatggg gctaatctgg 780
gagttgttac aggcgtctgc cttctgtgga cttggtttcc tgatagtcct tgccctttt 840
caggctgggc tagggagaat gatgatgaag tacagagatc agagagctgg gaagatcagt 900
gaaagacttg tgattacctc agaaatgatt gaaaatatcc aatctgttaa ggcatactgc 960
tgggaagaag caatggaaaa aatgattgaa aacttaagac aaacagaact gaaactgact 1020
cggaaggcag cctatgtgag atacttcaat agctcagcct tcttcttctc agggttcttt 1080
gtggtgtttt tatctgtgct tccctatgca ctaatcaaag gaatcatcct ccggaaaata 1140
ttcaccacca tctcattctg cattgttctg cgcatggcgg tcactcggca atttccctgg 1200
gctgtacaaa catggtatga ctctcttgga gcaataaaca aaatacagga tttcttacaa 1260
aagcaagaat ataagacatt ggaatataac ttaacgacta cagaagtagt gatggagaat 1320
gtaacagcct tctgggagga gggatttggg gaattatttg agaaagcaaa acaaaacaat 1380
aacaatagaa aaacttctaa tggtgatgac agcctcttct tcagtaattt ctcacttctt 1440
ggtactcctg tcctgaaaga tattaatttc aagatagaaa gaggacagtt gttggcggtt 1500
gctggatcca ctggagcagg caagacttca cttctaatga tgattatggg agaactggag 1560
ccttcagagg gtaaaattaa gcacagtgga agaatttcat tctgttctca gttttcctgg 1620
attatgcctg gcaccattaa agaaaatatc atctttggtg tttcctatga tgaatataga 1680
tacagaagcg tcatcaaagc atgccaacta gaagaggaca tctccaagtt tgcagagaaa 1740
gacaatatag ttcttggaga aggtggaatc acactgagtg gagaycaacg agcaagaatt 1800
tctttagcaa gagcagtata caaagatgct gatttgtatt tattagactc tccttttgga 1860
tacctagatg ttttaacaga aaaagaaata tttgaaagct gtgtctgtaa actgatggct 1920
aacaaaacta ggattttggt cacttctaaa atggaacatt taaagaaagc tgacaaaata 1980
ttaattttga atgaaggtag cagctatttt tatgggacat tttcagaact ccaaaatcta 2040

cagccagact ttagctcaaa actcatggga tgtgattctt tcgaccaatt tagtgcagaa 2100
agaagaaatt caatcctaac tgagacctta caccgtttct cattagaagg agatgctcct 2160
gtctcctgga cagaaacaaa aaaacaatct tttaaacaga ctggagagtt tggggaaaaa 2220
aggaagaatt ctattctcaa tccaatcaac tctatacgaa aattttccat tgtgcaaaag 2280
actcccttac aaatgaatgg catcgaagag gattctgatg agcctttaga gagaaggctg 2340
tccttagtac cagattctga gcagggagag gcgatactgc ctcgcatcag cgtgatcagc 2400
actggcccca cgcttcaggc acgaaggagg cagtctgtcc tgaacctgat gacacactca 2460
gttaaccaag gtcagaacat tcaccgaaag acaacagcat ccacacgaaa agtgtcactg 2520
gcccctcagg caaacttgac tgaactggat atatattcaa gaaggttatc tcaagaaact 2580
ggcttggaaa taagtgaaga aattaacgaa gaagacttaa aggagtgcct ttttgatgat 2640
atggagagca taccagcagt gactacatgg aacacatacc ttcgatatat tactgtccac 2700
aagagcttaa tttttgtgct aatttggtgc ttagtaattt ttctggcaga ggtggctgct 2760
tctttggttg tgctgtggct ccttggaaac actcctcttc aagacaaagg gaatagtact 2820
catagtagaa ataacagcta tgcagtgatt atcaccagca ccagttcgta ttatgtgttt 2880
tacatttacg tgggagtagc cgacactttg cttgctatgg gattcttcag aggtctacca 2940
ctggtgcata ctctaatcac agtgtcgaaa attttacacc acaaaatgtt acattctgtt 3000
cttcaagcac ctatgtcaac cctcaacacg ttgaaagcag gtgggattct taatagattc 3060
tccaaagata tagcaatttt ggatgacctt ctgcctctta ccatatttga cttcatccag 3120
ttgttattaa ttgtgattgg agctatagca gttgtcgcag ttttacaacc ctacatcttt 3180
gttgcaacag tgccagtgat agtggctttt attatgttga gagcatattt cctccaaacc 3240
tcacagcaac tcaaacaact ggaatctgaa ggcaggagtc caattttcac tcatcttgtt 3300
acaagcttaa aaggactatg gacacttcgt gccttcggac ggcagcctta ctttgaaact 3360
ctgttccaca aagctctgaa tttacatact gccaactggt tcttgtacct gtcaacactg 3420
cgctggttcc aaatgagaat agaaatgatt tttgtcatct tcttcattgc tgttaccttc 3480
atttccattt taacaacagg agaaggagaa ggaagagttg gtattatcct gactttagcc 3540
atgaatatca tgagtacatt gcagtgggct gtaaactcca gcatagatgt ggatagcttg 3600
atgcgatctg tgagccgagt ctttaagttc attgacatgc caacagaagg taaacctacc 3660
aagtcaacca aaccatacaa gaatggccaa ctctcgaaag ttatgattat tgagaattca 3720
cacgtgaaga aagatgacat ctggccctca gggggccaaa tgactgtcaa agatctcaca 3780
gcaaaataca cagaaggtgg aaatgccata ttagagaaca tttccttctc aataagtcct 3840
ggccagaggg tgggcctctt gggaagaact ggatcaggga agagtacttt gttatcagct 3900
tttttgagac tactgaacac tgaaggagaa atccagatcg atggtgtgtc ttgggattca 3960
ataactttgc aacagtggag gaaagccttt ggagtgatac cacagaaagt atttatttttt 4020
tctggaacat ttagaaaaaa cttggatccc tatgaacagt ggagtgatca agaaatatgg 4080
aaagttgcag atgaggttgg gctcagatct gtgatagaac agtttcctgg gaagcttgac 4140
tttgtccttg tggatggggg ctgtgtccta agccatggcc acaagcagtt gatgtgcttg 4200
gctagatctg ttctcagtaa ggcgaagatc ttgctgcttg atgaacccag tgctcatttg 4260

gatccagtaa cataccaaat aattagaaga actctaaaac aagcatttgc tgattgcaca 4320
gtaattctct gtgaacacag gatagaagca atgctggaat gccaacaatt tttggtcata 4380
gaagagaaca aagtgcggca gtacgattcc atccagaaac tgctgaacga gaggagcctc 4440
ttccggcaag ccatcagccc ctccgacagg gtgaagctct ttccccaccg gaactcaagc 4500
aagtgcaagt ctaagcccca gattgctgct ctgaaagagg agacagaaga agaggtgcaa 4560
gatacaaggc tttagagagc agcataaatg ttgacatggg acatttgctc atggaattgg 4620
agctcgtggg acagtcacct catggaattg gagctcgtgg aacagttacc tctgcctcag 4680
aaaacaagga tgaattaagt tttttttaa aaaagaaaca tttggtaagg ggaattgagg 4740
acactgatat gggtcttgat aaatggcttc ctggcaatag tcaaattgtg tgaaaggtac 4800
ttcaaatcct tgaagattta ccacttgtgt tttgcaagcc agattttcct gaaaaccctt 4860
gccatgtgct agtaattgga aaggcagctc taaatgtcaa tcagcctagt tgatcagctt 4920
attgtctagt gaaactcgtt aatttgtagt gttggagaag aactgaaatc atacttctta 4980
gggttatgat taagtaatga taactggaaa cttcagcggt ttatataagc ttgtattcct 5040
ttttctctcc tctccccatg atgtttagaa acacaactat attgtttgct aagcattcca 5100
actatctcat ttccaagcaa gtattagaat accacaggaa ccacaagact gcacatcaaa 5160
atatgcccca ttcaacatct agtgagcagt caggaaagag aacttccaga tcctggaaat 5220
cagggttagt attgtccagg tctaccaaaa atctcaatat ttcagataat cacaatacat 5280
cccttacctg ggaaagggct gttataatct ttcacagggg acaggatggt tcccttgatg 5340
aagaagttga tatgcctttt cccaactcca gaaagtgaca agctcacaga cctttgaact 5400
agagtttagc tggaaaagta tgttagtgca aattgtcaca ggacagccct tctttccaca 5460
gaagctccag gtagagggtg tgtaagtaga taggccatgg gcactgtggg tagacacaca 5520
tgaagtccaa gcatttagat gtataggttg atggtggtat gttttcaggc tagatgtatg 5580
tacttcatgc tgtctacact aagagagaat gagagacaca ctgaagaagc accaatcatg 5640
aattagtttt atatgcttct gttttataat tttgtgaagc aaaatttttt ctctaggaaa 5700
tatttatttt aataatgttt caaacatata ttacaatgct gtattttaaa agaatgatta 5760
tgaattacat ttgtataaaa taatttttat atttgaaata ttgacttttt atggcactag 5820
tatttttatg aaatattatg ttaaaactgg gacaggggag aacctagggt gatattaacc 5880
aggggccatg aatcaccttt tggtctggag ggaagccttg gggctgatcg agttgttgcc 5940
cacagctgta tgattcccag ccagacacag cctcttagat gcagttctga agaagatggt 6000
accaccagtc tgactgtttc catcaagggt acactgcctt ctcaactcca aactgactct 6060
taagaagact gcattatatt tattactgta agaaaatatc acttgtcaat aaaatccata 6120
catttgtgt 6129

<210> 6
<211> 1480
<212> PRT
<213> Homo sapiens

<400> 6

Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val Ser Lys Leu Phe
1 5 10 15

Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr Arg Gln Arg Leu
20 25 30

Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser Ala Asp Asn
35 40 45

Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu Ala Ser Lys
50 55 60

Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe Phe Trp Arg
65 70 75 80

Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val Thr Lys Ala
85 90 95

Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr Asp Pro Asp
100 105 110

Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile Gly Leu Cys
115 120 125

Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro Ala Ile Phe Gly
130 135 140

Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe Ser Leu Ile
145 150 155 160

Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp Lys Ile Ser
165 170 175

Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn Lys Phe Asp
180 185 190

Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro Leu Gln Val
195 200 205

Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala Ser Ala Phe
210 215 220

Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln Ala Gly Leu
225 230 235 240

Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly Lys Ile Ser
245 250 255

Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile Gln Ser Val
260 265 270

Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile Glu Asn Leu
275 280 285

Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr Val Arg Tyr
290 295 300

Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val Val Phe Leu
305 310 315 320

Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu Arg Lys Ile
325 330 335

Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala Val Thr Arg
340 345 350

Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu Gly Ala Ile
355 360 365

Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys Thr Leu Glu
370 375 380

Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val Thr Ala Phe
385 390 395 400

Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys Gln Asn Asn
405 410 415

Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu Phe Phe Ser Asn
420 425 430

Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn Phe Lys Ile
435 440 445

Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr Gly Ala Gly Lys
450 455 460

Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu Pro Ser Glu Gly
465 470 475 480

Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln Phe Ser Trp
485 490 495

Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr
500 505 510

Asp Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu
515 520 525

Asp Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly
530 535 540

Gly Ile Thr Leu Ser Gly Asp Gln Arg Ala Arg Ile Ser Leu Ala Arg
545 550 555 560

Ala Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser Pro Phe Gly
565 570 575

Tyr Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser Cys Val Cys

580 585 590

Lys Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser Lys Met Glu
595 600 605

His Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu Gly Ser Ser
610 615 620

Tyr Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln Pro Asp Phe
625 630 635 640

Ser Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe Ser Ala Glu
645 650 655

Arg Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe Ser Leu Glu
660 665 670

Gly Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln Ser Phe Lys
675 680 685

Gln Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile Leu Asn Pro
690 695 700

Ile Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr Pro Leu Gln
705 710 715 720

Met Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu Arg Arg Leu
725 730 735

Ser Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile Leu Pro Arg Ile
740 745 750

Ser Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg Arg Gln Ser
755 760 765

Val Leu Asn Leu Met Thr His Ser Val Asn Gln Gly Gln Asn Ile His
770 775 780

Arg Lys Thr Thr Ala Ser Thr Arg Lys Val Ser Leu Ala Pro Gln Ala
785 790 795 800

Asn Leu Thr Glu Leu Asp Ile Tyr Ser Arg Arg Leu Ser Gln Glu Thr
805 810 815

Gly Leu Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp Leu Lys Glu Cys
820 825 830

Leu Phe Asp Asp Met Glu Ser Ile Pro Ala Val Thr Thr Trp Asn Thr
835 840 845

Tyr Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile Phe Val Leu Ile
850 855 860

Trp Cys Leu Val Ile Phe Leu Ala Glu Val Ala Ala Ser Leu Val Val
865 870 875 880

Leu Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys Gly Asn Ser Thr
885 890 895

His Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr Ser Thr Ser Ser
900 905 910

Tyr Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp Thr Leu Leu Ala
915 920 925

Met Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr Leu Ile Thr Val
930 935 940

Ser Lys Ile Leu His His Lys Met Leu His Ser Val Leu Gln Ala Pro
945 950 955 960

Met Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile Leu Asn Arg Phe
965 970 975

Ser Lys Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro Leu Thr Ile Phe
980 985 990

Asp Phe Ile Gln Leu Leu Leu Ile Val Ile Gly Ala Ile Ala Val Val
995 1000 1005

Ala Val Leu Gln Pro Tyr Ile Phe Val Ala Thr Val Pro Val Ile Val
1010 1015 1020

Ala Phe Ile Met Leu Arg Ala Tyr Phe Leu Gln Thr Ser Gln Gln Leu
1025 1030 1035 1040

Lys Gln Leu Glu Ser Glu Gly Arg Ser Pro Ile Phe Thr His Leu Val
1045 1050 1055

Thr Ser Leu Lys Gly Leu Trp Thr Leu Arg Ala Phe Gly Arg Gln Pro
1060 1065 1070

Tyr Phe Glu Thr Leu Phe His Lys Ala Leu Asn Leu His Thr Ala Asn
1075 1080 1085

Trp Phe Leu Tyr Leu Ser Thr Leu Arg Trp Phe Gln Met Arg Ile Glu
1090 1095 1100

Met Ile Phe Val Ile Phe Phe Ile Ala Val Thr Phe Ile Ser Ile Leu
1105 1110 1115 1120

Thr Thr Gly Glu Gly Glu Gly Arg Val Gly Ile Ile Leu Thr Leu Ala
1125 1130 1135

Met Asn Ile Met Ser Thr Leu Gln Trp Ala Val Asn Ser Ser Ile Asp
1140 1145 1150

Val Asp Ser Leu Met Arg Ser Val Ser Arg Val Phe Lys Phe Ile Asp
1155 1160 1165

Met Pro Thr Glu Gly Lys Pro Thr Lys Ser Thr Lys Pro Tyr Lys Asn

1170 1175 1180

Gly Gln Leu Ser Lys Val Met Ile Ile Glu Asn Ser His Val Lys Lys
1185 1190 1195 1200

Asp Asp Ile Trp Pro Ser Gly Gly Gln Met Thr Val Lys Asp Leu Thr
1205 1210 1215

Ala Lys Tyr Thr Glu Gly Gly Asn Ala Ile Leu Glu Asn Ile Ser Phe
1220 1225 1230

Ser Ile Ser Pro Gly Gln Arg Val Gly Leu Leu Gly Arg Thr Gly Ser
1235 1240 1245

Gly Lys Ser Thr Leu Leu Ser Ala Phe Leu Arg Leu Leu Asn Thr Glu
1250 1255 1260

Gly Glu Ile Gln Ile Asp Gly Val Ser Trp Asp Ser Ile Thr Leu Gln
1265 1270 1275 1280

Gln Trp Arg Lys Ala Phe Gly Val Ile Pro Gln Lys Val Phe Ile Phe
1285 1290 1295

Ser Gly Thr Phe Arg Lys Asn Leu Asp Pro Tyr Glu Gln Trp Ser Asp
1300 1305 1310

Gln Glu Ile Trp Lys Val Ala Asp Glu Val Gly Leu Arg Ser Val Ile
1315 1320 1325

Glu Gln Phe Pro Gly Lys Leu Asp Phe Val Leu Val Asp Gly Gly Cys
1330 1335 1340

Val Leu Ser His Gly His Lys Gln Leu Met Cys Leu Ala Arg Ser Val
1345 1350 1355 1360

Leu Ser Lys Ala Lys Ile Leu Leu Leu Asp Glu Pro Ser Ala His Leu
1365 1370 1375

Asp Pro Val Thr Tyr Gln Ile Ile Arg Arg Thr Leu Lys Gln Ala Phe
1380 1385 1390

Ala Asp Cys Thr Val Ile Leu Cys Glu His Arg Ile Glu Ala Met Leu
1395 1400 1405

Glu Cys Gln Gln Phe Leu Val Ile Glu Glu Asn Lys Val Arg Gln Tyr
1410 1415 1420

Asp Ser Ile Gln Lys Leu Leu Asn Glu Arg Ser Leu Phe Arg Gln Ala
1425 1430 1435 1440

Ile Ser Pro Ser Asp Arg Val Lys Leu Phe Pro His Arg Asn Ser Ser
1445 1450 1455

Lys Cys Lys Ser Lys Pro Gln Ile Ala Ala Leu Lys Glu Glu Thr Glu
1460 1465 1470

Glu Glu Val Gln Asp Thr Arg Leu
1475 1480

INTERNATIONAL SEARCH REPORT

Inter[illegible]cation No
PCT/US 98/21887

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/35 A61K31/05 A61K31/375

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HWANG ET AL.: "Genistein potentiates wild-type and deltaF508-CFTR channel activity" AMERICAN JOURNAL OF PHYSIOLOGY, vol. 273, no. 3 Part 1, September 1997, pages C988-C998, XP002093436 see page C997, column 2	1-3, 5-13, 24-26
Y	--- -/--	14, 16-23, 27-29, 51,52,57

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 February 1999	02/03/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Alvarez Alvarez, C

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCOTT ET AL: "Ascorbic acid stimulates chloride transport in the amphibian cornea" INVESTIGATIVE OPHTALMOLOGY, vol. 14, no. 10, October 1975, pages 763-766, XP002093437 see table 1	4,15
Y		21-23, 29,57
Y	SMITH: "Treatment of cystic fibrosis based on understanding CFTR" J. INHER. METAB. DIS., vol. 18, 1995, pages 508-516, XP002064542 see page 510 "Restoration of trafficking of mutant CFTR"	14, 16-20, 27,51,52
Y,P	RUBENSTEIN ET AL.: "In vitro pharmacologic restoration of CFTR-mediated chloride transport..." JOURNAL OF CLINICAL INVESTIGATION, vol. 100, no. 10, November 1997, pages 2457-2465, XP002076381 see page 2464 "Conclusions"	14, 16-19, 27,51,52
Y	RANDELL BROWN ET AL.: "Chemical chaperones correct the mutant phenotype of the deltaF508 cystic fibrosis..." CELL STRESS & CHAPERONES, vol. 1, no. 2, 1996, pages 117-125, XP002093438 see abstract see "Discussion"	18,20, 28,51,52
X	DATABASE WPI Week 8715 Derwent Publications Ltd., London, GB; AN 87-105816 XP002093439 & JP 62 053923 A (TEIJIN LTD.) , 9 March 1987 see abstract	54
X	DATABASE WPI Week 9342 Derwent Publications Ltd., London, GB; AN 93-330545 XP002093440 & JP 05 236910 A (NISSHIN FLOUR MILLING CO.), 17 September 1993 see abstract	57

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 9434 Derwent Publications Ltd., London, GB; AN 94-277493 XP002093441 & RU 2 008 015 C (MAKSYUTINA) , 28 February 1994 see abstract	57
X	DATABASE WPI Week 9518 Derwent Publications Ltd., London, GB; AN 95-135875 XP002093442 & JP 07 059548 A (YAEGAKI HAKKO GIKEN KK) , 7 March 1995 see abstract	57
A	SHEPPARD ET AL.: "Mutations in CFTR associated with mild-disease-form chloride channels with altered pore properties" NATURE, vol. 362, 11 March 1993, pages 160-164, XP000612158 see the whole document	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Ir.. .national application No.
PCT/US 98/21887

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)